STUDIES IN ATYPICAL FORMS OF TUBERCLE BACILLI ISOLATED DIRECTLY FROM THE HUMAN TISSUES IN CASES OF PRIMARY CERVICAL ADENITIS.[1]

With Special Reference to the Theobald Smith Glycerine Bouillon Reaction.

By CHARLES W. DUVAL, M.D.

Professor of Pathology, Tulane University, New Orleans.

(From the Pathological Laboratory of the Montreal General Hospital.)

Plates XIV and XV.

INTRODUCTION.

Koch's statement in 1901 (1) that bovine tubercle bacilli are quite incapable of infecting human beings has led to much careful research being devoted to the identification of the type of tubercle bacilli which can be derived from tuberculous lesions in man. As one of the criteria of type the glycerine-bouillon test introduced by Theobald Smith (2) in 1905 has been used to separate the human from the bovine type of the bacillus. It has now been established that the bovine bacillus can be implanted upon and grown in human tissues where they produce lesions of tuberculosis. My own studies have been devoted to the identification of the types of tubercle bacilli derived from cultures from a small series of cases of primary cervical adenitis. The clinical course of the disease in the several instances was quite similar while the tubercle bacilli isolated and studied carefully over a period of two years have proven to be distinct from one another. On the other hand, the clinical appearances observed in these cases were quite different from those usually observed in tuberculous cervical adenitis and led directly to my undertaking a study of the bacilli yielded by the lesions.

The four cultures which form the basis of this communication

[1] Paper read before the International Tuberculosis Conference held at Washington, D. C., October, 1908. Received for publication November 14, 1908.

were studied with the view of determining, if possible; (1) the type of infection; (2) whether the growth features, reaction changes in glycerine bouillon, and virulence for rabbits and guinea-pigs, remain relatively constant for a period extending over years; (3) if transitory changes occur in virulence, growth and reaction in bouillon, which might explain the so-called "intermediate" types of bovine and human cultures which have been described; (4) whether the T. Smith glycerine-bouillon test serves to determine degrees of adaptation in man to bacilli of the lower host-species.

A pure culture was obtained in each case by transplanting portions of the infected tissues from the neck to a modified egg medium compounded and prepared as follows:

> The yolks are carefully separated from the whites of twelve eggs and each dropped separately and aseptically into a flask containing 100 c.c. of human blood serum, 200 c.c. of distilled water and 10 c.c. of glycerine which has already been sterilized by the intermittent method of sterilization (Arnold). The flask is now thoroughly shaken and the mixture carefully tubed. These tubes are heated in the slanted position at a temperature not exceeding 70° C. until the consistence of the medium is sufficiently firm to insure its holding together when the tubes are in the upright posture. It is essential to obtain the egg mixture sterile in the test tubes before they are inspissated so that the one heating, which is necessary to solidify the medium, suffices. This gives a soft golden yellow medium with plenty of water of condensation. The cotton plugs are paraffined to keep the medium moist.

From the original cultures a growth upon glycerine bouillon was initiated by depositing upon sterile paper "floats" masses of egg culture. Further, guinea-pigs were inoculated subcutaneously with tuberculous material from the neck. After the tuberculous process was set up portions of the caseous glands were planted upon the modified egg medium. The growth thus obtained was transferred to glycerine bouillon by the method described above.

After the initial isolation, observations upon growth features, reaction changes in glycerine bouillon, and virulence for rabbits and guinea-pigs were observed as to their relative constancy over an extended period.

The glycerine bouillon tests were carried out with the following technique: three to six siphon-flasks were simultaneously inoculated from the same culture and controlled by similar transplants of human, bovine and avian standard cultures of the same age and grown upon the same medium.

Titrations were begun from the new cultures and the controls as soon as peripheral growth appeared about the floating transplants. It should be noted that before inoculation all the bouillon, which was prepared in the usual way from fresh muscle infusion, was accurately standardized and the percentage of acidity of each carefully tabulated. Throughout the experiments 500 c.c. flasks were employed in order to provide for the waste by titration and loss by evaporation and to make possible an accurate comparison of the inhibitive influence of the growth products of the different cultures. Titrations were repeated at intervals of one to two weeks over a period of six to twelve months. The quantity for all titration tests was 5 c.c. of the bouillon culture medium in 45 c.c. of distilled water. The initial acidity of the glycerine bouillon was varied, and a series of 1, 1.5, 2, 2.5 and 3 per cent. acidity was observed.

The virulence of the new cultures with relation to growth variations was determined from time to time and compared with the virulence of the standard type cultures by subcutaneous and intravenous inoculations of rabbits and guinea-pigs. The dosage was standardized as far as possible by weighing and emulsifying in physiological salt solution equal quantities of growth material from the various cultures.

THE CLINICAL CASES.

In all the cases the tuberculous process manifested itself as a rapidly increasing swelling of the neck, which in every way simulated an acute pyogenic infection. It was necessary in two of the patients to perform a tracheotomy to relieve the respiratory distress. A careful physical examination failed to show any signs elsewhere in the body of tuberculosis.

Three of the cases died of acute disseminated miliary tuberculosis in a few weeks after the first symptoms of cervical enlargement was noted. The fourth case was treated with tuberculin and apparently made a complete recovery.

The three autopsies showed a primary tubercular focus in the cervical lymph glands from which resulted acute general miliary tuberculosis.

The following are brief clinical notes and abstracts from the autopsy protocols:

CASE I. *Clinical History.*—Mrs. L., 49 years old, a native of Canada, who lived on a farm all her life, entered the Montreal General Hospital June 4, 1906. Three weeks before admission she noticed for the first time a swelling on the left side of the neck, which rapidly increased in size during the next five days when it ruptured and discharged. In two weeks the swelling had spread over the entire left side of the neck from the lower jaw to the clavicle. At no time did the mass cause pain or give rise to tenderness on pressure.

Following incision a stained smear preparation of the pus was crowded with acid-fast bacilli. The bacilli occurred for the most part in closely aggregated masses, and in order to obtain them sufficiently scattered for a microscopic study it was necessary to dilute one part of the pus in three parts of water. The thinner parts of the expressed material contained myriads of pin-head and smaller sized reddish-brown granules which when crushed and stained proved to be pure masses of the bacilli.

A small quantity of the pus was transplanted upon modified egg-medium and also upon the ordinary culture media and incubated at 37° C. The egg mixture after four or five days became thickly studded with discrete colonies 1 mm. in size which proved to be acid-fast bacilli. All the inoculated tubes of ordinary media remained sterile, a fact especially significant in view of the necrotic condition of the tissues and the presence of an open avenue for secondary infection.

Full grown guinea-pigs and rabbits were inoculated subcutaneously and intraperitoneally with 0.5 c.c. each of the pus from the neck. The guinea-pig and rabbit that received intraperitoneal injections died within fifteen days of generalized tuberculosis. The rabbit inocluated subcutaneously died on the twenty-sixth day. The autopsy revealed an advanced tuberculosis; the lungs, liver and spleen showing the most extensive involvement. The guinea-pig similarly inoculated died on the fourteenth day of disseminated tuberculosis. Pure cultures of tubercle bacilli were recovered directly upon modified egg-medium from the spleen of each animal.

From the date of admission the patient failed rapidly and died five days later from acute general miliary tuberculosis; or four weeks after the first appearance of the disease.

Autopsy.—Sixteen hours post-mortem. Body of a well nourished woman. The whole left side of the neck is occupied by a large nodular mass which extends from the lower jaw to the clavicle and from the trapezius muscle to the median line in front. Over the tumor are three linear incisions.

The peritoneum and plurae are beset with disseminated miliary tubercles.

The lungs are crepitant throughout and everywhere beset with innumerable discrete tubercles. No evidence of old tuberculous foci.

The other organs aside from acute miliary tubercles show nothing remarkable.

A pure culture of *B. tuberculosis* was isolated from the heart's blood on egg-medium; twelve or more colonies developing from six platinum loopfuls of the blood.

Anatomical Diagnosis.—Primary tuberculosis of the right cervical lymph glands; acute general miliary tuberculosis; *B. tuberculosis septicæmia.*

The tuberculosis in this case was undoubtedly primary in the

cervical glands, for a most careful search at autopsy failed to reveal any older focus.

In the human tissue the tubercle bacilli were quite long and slender and transversely segmented. They retained Gram's stain readily. Indeed, even in tissue-sections fixed in Zenker's fluid, embedded in paraffin and treated in the usual routine manner with eosin-methylene-blue the bacilli were stained intensely with eosin.

The bacilli were so numerous in the sections of the human spleen and lymph nodes that it was difficult to discern clearly the nuclei of the tissue cells even in fields where there was no necrosis. The organisms occurred without definite arrangement. Frequently the bacilli were packed so closely together that whole fields of the microscope consisted of one dense layer of slender, beaded rods (see Fig. 1, Plate XIV). In those parts of the sections where the bacilli were less dense, large numbers of them appeared within mononuclear leucocytes. These engorged cells were analogous to the phagocytes for red blood cells, so common in the lymphoid structures in typhoid fever. One could frequently count fifty or more bacilli within a single cell. The descriptions of occurrence of enormous numbers of leprosy bacilli in human tissues are inadequate to convey a correct idea of the picture here presented in tuberculosis. Lydia Rabinowitsch (3), however, describes a similar distribution of bacilli and phagocytic cells in tuberculous tissues of fowls.

The bacilli when cultivated on artificial medium presented an entire change in morphology from that observed in the tissues; instead of the long slender beaded rods appeared small plump bacilli almost coccal in character, and without a suggestion of transverse massing of the chromatin. On egg-medium the first generation presented a most curious arrangement of the bacilli, which even the manipulations with the platinum loop failed to disturb. The individual rods occurred one behind the other in parallel rows forming broad wavy bands that extended across many fields of the microscope. This end-to-end arrangement of the individual organisms is most unusual in tubercle bacilli and resembles the appearance met with in anthrax colonies.

The character of the growth on ordinary media employed for the cultivation of tubercle bacilli completely corresponds with the

human type of culture. The growth was always very dry. Though sometimes fine and film-like the culture at other times was luxuriant, lumpy and of wax-like whiteness. It never presented the "moist appearance" that is said to be characteristic of avian cultures. A feature of the bacillus was its prolonged viability on artificial media, a profuse growth having been obtained from the original culture, which had been sealed and kept corked at room temperature for more than two years. Lydia Rabinowitsch (3) claims that prolonged viability is a distinguishing feature of the avian bacillus; in some instances under refrigeration the culture remaining alive for two years. Culture I not only remained viable for a period of years at ordinary temperature but retained its virulence for rabbits and guinea-pigs.

The results of animal inoculation with Culture I afforded a complete illustration of the possible variation in the histological lesion. There was a distinct difference between the microscopic character of the human lesion and that produced experimentally. The subacute inflammatory type of lesion in man, which also occurred in the earlier experimental animals, was a striking feature, and would indicate an extremely high degree of virulence. The lesion in man consisted chiefly of lymphoid and plasma cells and large numbers of polymorphonuclear leucocytes, with only an occasional epithelioid type of cell and no giant cells. In the experimental lesion produced by the culture somewhat attenuated in virulence the epithelioid elements with central necrosis and giant cells predominated. Giant cells did not occur in the earlier rabbits and guinea-pigs inoculated, nor in the lesions of the chickens. In later experiments, however, the animal tissues showed numbers of giant cells associated with the lesions. It would seem that the occurrence of giant cells in tuberculosis, as in glanders (4), depends more on the virulence of the organism than on any other factor. This culture at least suggests that the type of lesion, whether exudative or proliferative, is due to the number and virulence of the bacilli.

Lydia Rabinowitsch (3) noted in avian tuberculosis, distinctive differences in the histological picture. In the fowl the alveoli of the lungs were filled with an exudate composed of epithelioid and lymphoid cells, together with numerous giant cells containing large numbers of the tubercle bacilli. This author attaches

special significance to these large cells filled with an enormous number of bacilli and regards them as characteristic of avian tuberculosis. This same histological picture is seen in the human and experimental lesions produced by Culture I. Here the lymphoid and large mononuclear cells were crowded with bacilli and presented an analogous picture in this respect to leprosy.

The culture, after it had become attenuated in virulence, produced, after repeated intravenous injection, a lesion in the glands of the rabbit which exhibited a certain histological resemblance to that of Hodgkin's disease such as Dorothy Reed (5), Longcope (6) and others have described.

The virulence of the culture was tested for rabbits and guinea-pigs, the animals being approximately of the same age and weight. Chickens, pigeons and rats were also used. Each comparative test was made intravenously, intraperitoneally and subcutaneously with rabbits, guinea-pigs and pullets. One milligramme of culture given intraperitoneally produced a fatal tuberculosis in these animals in four to six weeks. The same dose administered subcutaneously required a much longer time to produce the same result and in some instances both guinea-pigs and rabbits lived for months. Ten milligrammes, however, when injected subcutaneously, gave rise to disseminated tuberculosis in six to ten weeks.

A series of six chickens, three hens and three pullets, received each one milligramme of the culture intraperitoneally, subcutaneously and intravenously. Six weeks later the fowls were killed and the two pullets which had received intraperitoneal and intravenous injections showed well-marked lesions of tuberculosis. The mesentery of the intraperitoneally injected pullet presented numerous discrete 2 mm. sized tubercles (see Fig. 4, Plate XV). No lesions in the other organs were noted. The pullet which received an intravenous injection showed a number of small tubercular nodules in the lungs and spleen. The subcutaneously inoculated pullet and the three hens presented no trace of tuberculosis. When we consider the small dosage and the short period allotted the chickens to live the occurrence of macroscopic lesions is most remarkable. Larger doses invariably produced lesions in the half-grown chickens which as Bang (7) claims are far more susceptible than full-grown fowls. Positive results occurred in chickens following intraperitoneal inocu-

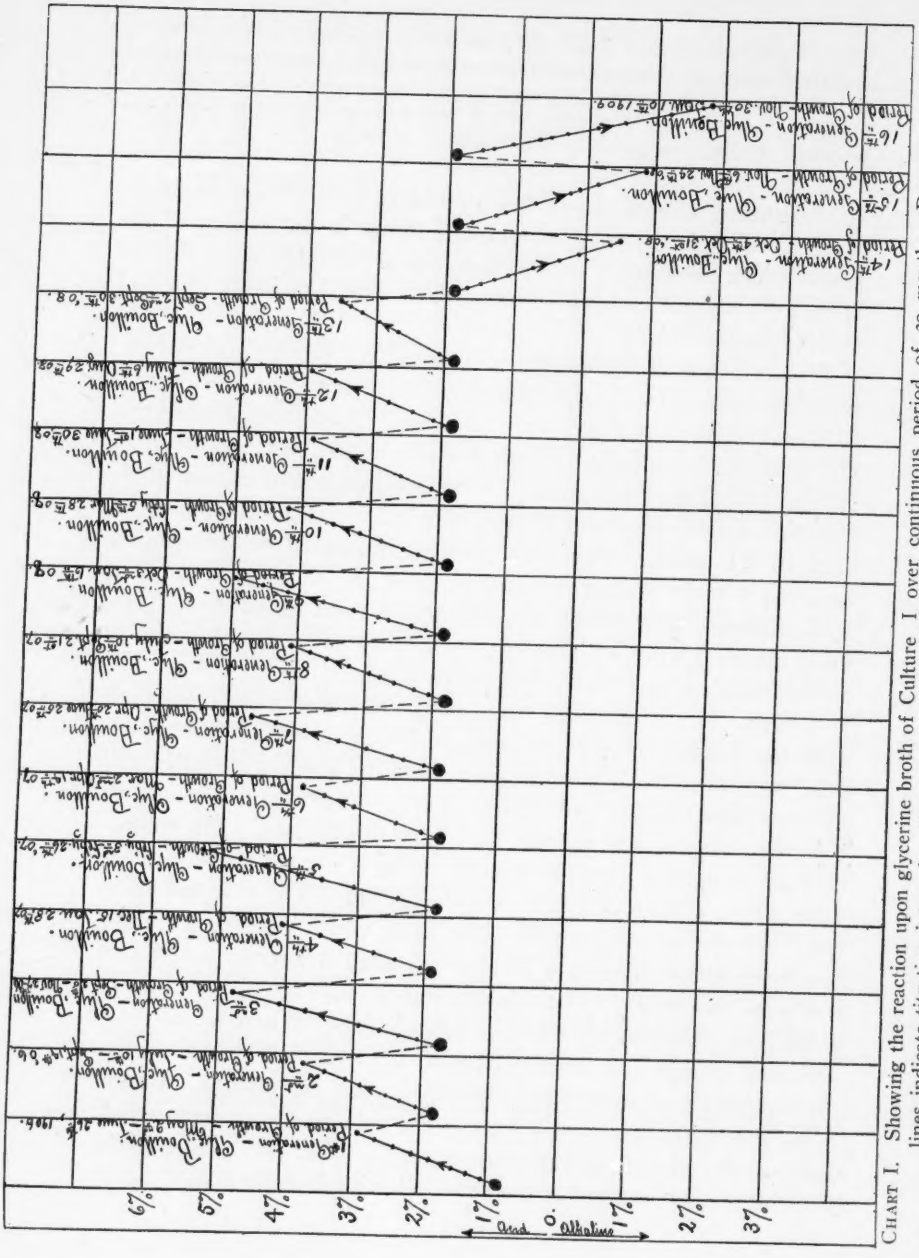


CHART I. Showing the reaction upon glycerine broth of Culture I over continuous period of 30 months. Dots upon arrowed lines indicate titration intervals. Note the complete change in reaction for the fourteenth generation.

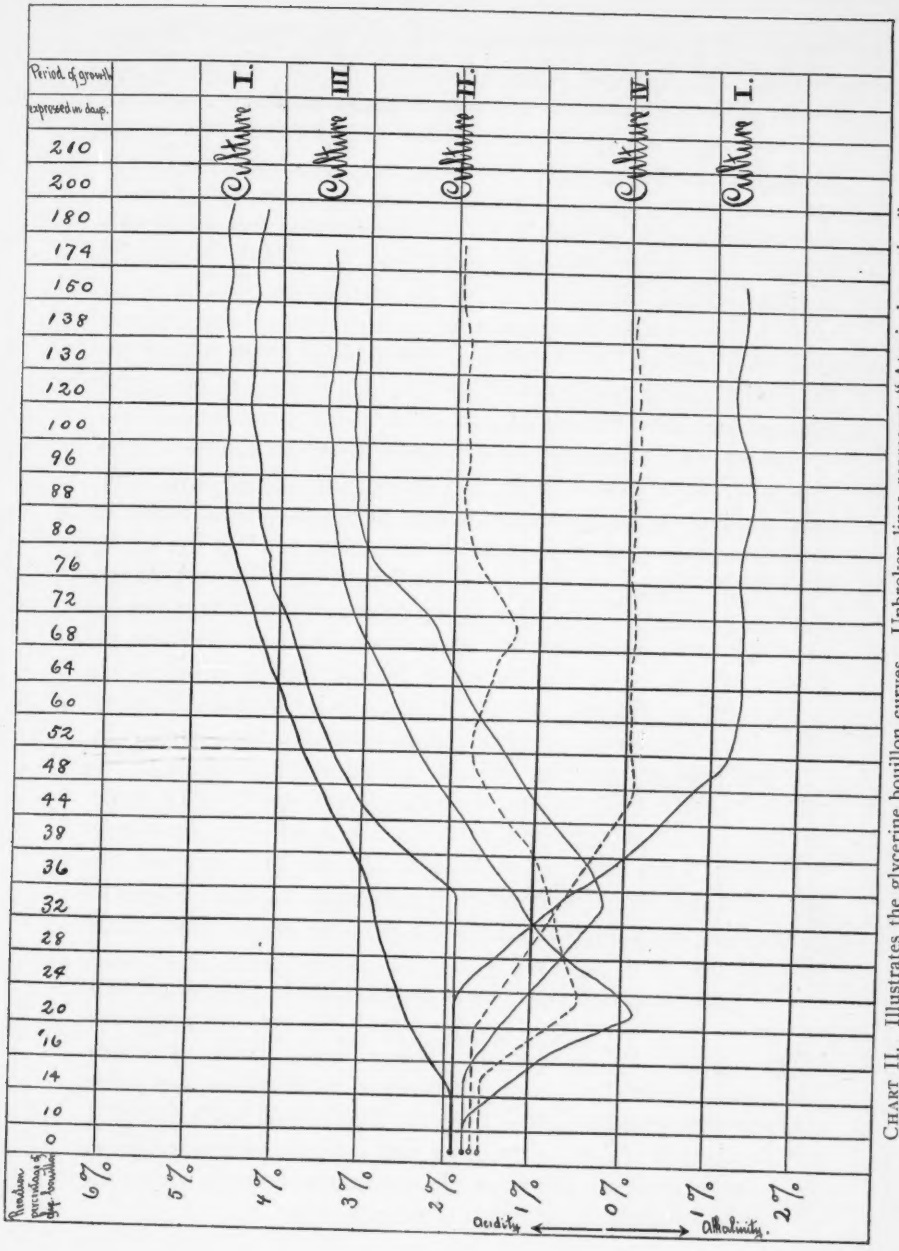


CHART II. Illustrates the glycerine bouillon curves. Unbroken lines represent "Atypical reaction" curves.

lation with greater frequency than for any other method of introduction. As controls, fowls were injected with bovine IX and human XII (Theobald Smith) with negative results.

The reaction curve for this culture in glycerine bouillon was decidedly atypical for more than two years. It always gave an increase in the percentage of acidity without the initial fall that is so characteristic of all tubercle cultures. The reaction was occasionally delayed for weeks after the growth had completely covered the bouillon surface (a feature in my experience not peculiar to any strain), but when the change in reaction began there always occurred a constant rise until an elevation of five or more points higher than the initial acidity was attained, after which there was no further change (see chart, Culture I).

For a period of more than two years after the culture was recovered from the human tissue the behavior towards glycerine broth was certainly unlike any known human or bovine type of organism since invariably it failed to produce the "initial fall" in the acidity percentage. Again during this period the culture did not behave in respect to glycerine bouillon as the control avian culture (Smith 2) which as O. Bang (8) first pointed out, describes the same reaction curve as the bovine, namely, a gradual decline in percentage acidity until an alkaline reaction results. Quite recently, however, this culture has given a complete change in the reaction curve.

It has been my practice each month since the isolation of the culture to make fresh transplants upon glycerinated bouillon to determine whether subsequent generations might not show a variation in reaction. I was therefore surprised to discover after two and one-half years that the culture suddenly underwent a complete reversal in its action upon glycerine producing now the typical curve of the avian bacilli (see Chart I).

The organism from this case was regarded at first as a modified avian bacillus or an "intermediate" between the avian and human types. Its prolonged viability under artificial conditions and the production of tuberculosis in the fowl are points indicating an avian histogenesis, but the former high acid production and the absence of moist growth on media do not correspond to the typical avian bacillus. However, the recent behavior upon glycerine bouillon leaves little doubt that it is avian in type.

Case II. *Clinical History.*—Samuel B., 20 years old, a native of Ireland, entered the Montreal General Hospital in October, 1906, complaining of shortness of breath and great weakness. Two weeks before admission he had noticed for the first time a swelling on the right side of the neck, which rapidly increased until it attained approximately the size of an orange.

The swelling was opened under surgical precautions and a large amount of creamy yellow material removed. A portion of this was immediately spread over modified egg medium which after three weeks at 37° C. developed a pure culture of the tubercle bacillus. The ordinary culture media inoculated at the same time remained sterile. Stained smear preparations of the pus from the neck showed only a moderate number of short, thin, acid-fast bacilli. The first growth on artificial media was not luxuriant and rather slow in development, but there was no difficulty in getting transplanted "floats" from the original culture to grow on glycerine bouillon. Pus was injected intraperitoneally and subcutaneously into rabbits and guinea-pigs. The rabbits which received an intraperitoneal inoculation died six weeks later of generalized tuberculosis, while the one which had been injected subcutaneously, when killed four months afterwards, showed only tuberculosis about the site of inoculation and small nodules in the lungs. Both guinea-pigs died of tuberculosis; the intraperitoneal animal in five weeks and the subcutaneous one in nine weeks.

Autopsy.—Fifteen hours after death. Subject emaciated. On the right side of the neck there is a swelling that extends from the jaw downward and behind the clavicle. Over this area is an incision 3 cm. in length which exposes a large mass of yellowish-white necrotic material. On opening the mass it is found to be continuous with another in the anterior mediastinum which is approximately 12 cm. by 16 cm. in extent and completely encircles the trachea. The other superficial lymphatic glands of the body are not enlarged.

All the organs contain miliary tubercles.

Anatomical Diagnosis.—Glandular tuberculosis; acute miliary tuberculosis of the liver, spleen and kidneys; acute tuberculous pleuritis.

The organism recovered from this case—designated Culture II—belongs undoubtedly to the human group of tubercle bacilli. Though the growth was always slow to develop on glycerine bouillon the culture corresponds in other features, especially virulence for rabbits, to the human variety—and further, while the bio-chemical reaction as interpreted by glycerine bouillon titrations showed at times aberrations from the normal, the curve furnished by this organism was found on the whole to tally closely with the human type.

Case III. *Clinical History.*—Francesco B., 24 years old, entered the Montreal General Hospital in October, 1906, complaining of a swollen neck and difficulty in breathing and swallowing. Personal history negative. There were fluctuating masses on both sides of the neck extending from the mastoid processes to the clavicles.

The third day after admission the mass on the right side of the neck was incised and 16 ounces of thick creamy pus and caseous material removed. The

mass on the left side was opened on the following day and 8 ounces of pus evacuated. A portion of the pus was transferred to egg medium which showed a pure growth of tubercle bacilli after ten days' incubation.

A pair of guinea-pigs and rabbits were inoculated subcutaneously and intraperitoneally with a portion of an emulsion prepared from the discharge. The rabbits were killed, one four weeks, the other three months after inoculation. The intraperitoneally injected animal showed no trace of tuberculosis while the one which was subcutaneously injected contained definite tuberculous lesions at the site of injection and in the glands of the groin. The guinea-pigs injected had increased in weight. The superficial inguinal glands of the subcutaneously inoculated animal were enlarged and contained infarcts and caseous nodules. In the intraperitoneally inoculated pig the only tubercular lesion presented itself in the omentum. The cultures recovered from these animals in no way differed from the one obtained directly upon artificial medium from the human tissues.

The patient died on October 31 of sudden cardiac failure, five weeks after the first symptoms were noted.

Autopsy.—Thirteen hours post-mortem. On either side of the neck, which is enormously swollen, there is a deep incision 3 cm. in length, the edges of which are gangrenous.

Peritoneal Cavity.—The omentum and intestines are glued together by recent fibrinous adhesions. The parietal peritoneum is studded with glistening tubercles. The mesenteric lymph nodes are enlarged and caseous.

The lungs, spleen, liver, kidneys and brain are thickly studded with minute pinpoint translucent tubercles. No old lesion in the lungs.

Cæcum, descending part of the colon and the lower part of the ileum contain numerous tuberculous ulcers.

Anatomical Diagnosis.—Primary tuberculosis of the cervical glands; acute general miliary tuberculosis; acute ulcerative entero-colitis.

A comparative study of the culture isolated from this case—designated Culture III (atypical)—corresponds with the human variety of tubercle except in regard to its behavior at times upon glycerine broth where it yields a neutral or 0.5 per cent. alkaline reaction before the rise in acidity is established.

The culture possesses a low degree of virulence for guinea-pigs and rabbits. Full-grown rabbits seldom developed tuberculosis following intraperitoneal injections of ten milligrammes of fresh culture. Adult guinea-pigs inoculated intraperitoneally with the same amount frequently increased in weight and when killed three months afterwards showed only a few nodules in the omentum.

Case IV. *Clinical History.*—Antonio C., aged 16, entered the Montreal General Hospital in March, 1908, complaining of a swollen neck. The cervical glands are enormously enlarged on both sides of the neck and range in size from a marble to that of a hen's egg. They are discrete, freely movable and give no tenderness on pressure. The mediastinal glands on percussion are found greatly

enlarged and apparently continuous with the cervical mass. The axillary, inguinal and other superficial glands of the body are not enlarged, but the abdominal glands can be distinctly palpated. The heart and lungs are normal. One week after entrance to the wards the swelling of the neck greatly increased in size and became less nodular in character; instead of freely movable, discrete masses there is now a solid tumor which extends from the jaw downward under the clavicles and into the mediastinum. The swelling was opened under aseptic precautions and a gland 2 by 1 cm. in diameter removed for bacteriological study. On section it was firm and contained large yellowish-white areas; no bacilli, however, were demonstrable in the stained smears. Cultures were obtained directly upon egg medium from transplanted pieces which were excised from the central part of the gland. The ordinary laboratory media inoculated remained sterile. The egg medium showed the first sign of growth after three weeks when it became studded with small colonies of the tubercle bacillus.

Soon after admission the patient was given at regular intervals in gradually increasing doses T.R. (tuberculin), under which treatment the cervical enlargement completely subsided. The glandular mass in the neck began to diminish in size from the first dose. Those in the mediastinum and the abdomen also disappeared. The patient gained markedly in weight and the temperature fell to and remained normal. He was discharged apparently well, one month after the first administration of the tuberculin.

The culture from this case, designated IV, completely corresponds in respect to growth and reaction upon glycerine bouillon to the standard bovine type; however, it does not correspond in degree of virulence for rabbits. Fifty milligrammes of fresh culture emulsified in 5 c.c. of sterile salt solution and injected intravenously into the ear of the rabbit failed repeatedly to produce lesions. Rabbits inoculated subcutaneously with the same dosage did not develop disseminated tuberculosis; and when killed months afterwards presented lesions only at the site of inoculation or in the regional lymph glands. On the other hand, guinea-pigs injected intraperitoneally with ten milligrammes of the culture rapidly developed generalized tuberculosis. As yet I have not tested the virulence of the culture for calves. In respect to growth the culture always manifested a tendency to develop slowly. Though under artificial cultivation for nearly a year it still refuses to grow on glycerine agar. On glycerinated bouillon growth increases very slowly though always as a heavy colony-mass and at no time film-like which is so often the case in feebly growing bovine cultures. For the present, at least, I regard Culture IV as an atypical bovine variety of the tubercle bacillus possessing a low degree of virulence for rabbits. Atypical

cultures producing a neutral or slightly alkaline reaction upon glycerine broth have been described by Theobald Smith (9) and O. Bang (8) and regarded by them as "atypical" human rather than bovine in type; however, the slow multiplication of Culture IV on the more favorable media and the inability to grow at all on glycerine agar, together with the fact that it produces a neutral or slightly alkaline reaction are factors strongly in favor of a bovine nature.

THE GLYCERINE BOUILLON REACTION AS A DIFFERENTIAL TEST.

Theobald Smith (10) in 1905 described the difference in behavior of "bovine" and "human" bacilli towards glycerine bouillon which corresponded with the author's previously recognized variation in virulence. Before the glycerine broth test was known, however, the differentiation of these depended mainly on virulence and rapidity of growth. The feeble and slowly growing organism if highly virulent for rabbits when injected intravenously, was regarded as bovine in type; while the luxuriant and rapidly growing culture that possessed a low degree of virulence for rabbits was classed as the human variety.

In my experience the glycerine bouillon test is more reliable than "degree of virulence" in the differentiation of mammalian strains provided it is repeated over a long period of time. Even with regard to avian cultures O. Bang (8) claims that the alkalinity produced in glycerine broth is so much greater than with the bovine type that it serves to distinguish between them. My observations upon the behavior of Culture I are in accord with this finding.

The test, which consists of growing the organism on the surface of glycerine bouillon and titrating from time to time to note the change in reaction, not only divides the tubercle family into two distinct groups but it may be a qualitative means of detecting bacilli of the modified bovine type in the human body or the degree of adaptation. Though the method does not sharply differentiate between bacilli of the lower host-species it is of aid in the identification of these lower type forms in the human body. Difference in virulence and character of growth are too variable to allow us to admit unreservedly that one type may become transformed into another through animal passage. These properties even with the

same culture, vary within wide limits, more especially in the growth features which depend so largely on the nature of the culture medium. Yet it would seem that these modifications in biological and pathogenic characters have been the crucial tests employed by those who claim to have transformed the avian into the bovine type of bacillus.

L. Rabinowitsch (3) in a study of 95 avian cultures noted a whole growth scale from the typical moist shiny growth of the avian to the dry scaly appearance of the mammalian type of bacillus, and is most emphatic that from three birds she isolated cultures that were identical with the human type of bacillus. She also claims to have completely transformed a typical avian culture into the human type of bacillus by repeated passage through animals. In view of the fact that she draws particular attention to the inconstant and interchangeable differences in growth and virulence of the mammalian and avian bacilli and that in morphology and staining reaction there is no essential variation, it is hard to understand how "atypical" forms were definitely determined, especially as there is no mention of the glycerine bouillon reaction. If we regard the tubercle bacilli of the different host-species as variations of the same family the existence of "intermediate forms" is not only possible but highly probable. There is reason to believe that aberrant types of the tubercle bacillus exist as they do for other bacterial species, but there is as yet no absolute test by which the finer differences may be determined. Our present methods at best are too inadequate to permit us to state positively with regard to "intermediate" forms of the tubercle bacillus. Some so-called atypical cultures that have at first behaved unlike the standard types if repeatedly tested over a longer period of time might eventually fall into the well-known groups. The most striking examples of this change have occurred with Cultures I and III of this series.

It would seem that these strains have only recently accepted a new environment and are but slightly altered by the host influence. In this way it is possible to explain their odd behavior upon glycerine broth after a year's sojourn outside the animal body. For more than a year Culture III, though constantly under cultivation and repeatedly tested, always approached, but never reached, the neutral

TABLE I.

Showing the Reaction of Glycerine Bouillon for Cultures under Different Temperature Conditions.

Bouillon.	Case	Culture.	Source.	Virulence.	Inoculation Date.	Titration. Dates.		Reaction. Per cent. Acidity.	Remarks.
Beef Inf. 1000 c.c. Peptone 10 grams. Glycerine 40 c.c. Acidity 1.9 per cent.	Mrs. S., age 49, native of Canada. Primary cervical adenitis with fatal tuberculosis septicæmia.	I (Flask "a"). "Aptypical" organism with biochemical reactions resembling avian bacilli.	Pus from cervical region. Direct isolation from the human tissue upon modified egg medium.	Equally virulent for rabbits and guinea-pigs. Chickens are also susceptible.	March 24, 1908.	Apr. 18, '08	Period under Incubator temp.	1.9	Heavy growth covering three-quarters of bouillon surface.
						" 25, "		2.2	Heavy growth covering whole of bouillon surface.
						May 10, "		2.8	Growth completely over surface and up sides of flask.
						" 20, "		3.0	Growth completely over surface and up sides of flask.
						June 1, "		3.2	No change in growth. Flask removed from incubator.
						" 14, "	Room temp.	3.5	Growth at bottom of flask. Bouillon perfectly clear.
						July 4, "		5.0	Growth at bottom of flask. Bouillon perfectly clear.
						Aug. 6, "		5.0	Growth at bottom of flask. Bouillon perfectly clear.
Ditto.	Ditto.	I (Flask "b").	Ditto.	Ditto.	March 24, 1908.	Apr. 18, "	Period under Incubator temp.	1.9	Growth heavy, film covering one-half bouillon surface.
						" 25, "		1.9	Growth heavy, film covering three fourths bouillon surface.
						May 10, "		2.2	Growth over whole bouillon surface.
						" 20, "		2.8	Growth complete and up sides of flask.
						June 1, "		3.0	Growth complete and up sides of flask
						" 14, "		3.1	Growth allowed to remain on surface of flask.
						July 4, "		4.2	Growth intact over surface of bouillon.
						Aug. 6, "		4.2	Growth intact over surface of bouillon.
Beef Inf. 1000 c.c. Peptone 10 grams. Glycerine 40 c.c. Acidity 2.0 per cent.	Franc. B., age 24, native of Italy. Primary cervical adenitis resulting in fatal acute miliary tuberculosis.	III (Flask "a"). "Atypical" unusual reaction in glyc. bouillon.	Pus from cervical region. Direct recovery of culture from the human tissue upon modified egg medium.	Moderately virulent for rabbits. Highly virulent for guinea pigs Avirulent for chickens.	March 13, 1908.	Apr. 18, "	Period under Incubator temp.	2.0	Growth size of half dollar.
						" 30, "		2.0	Growth covers one-half of surface.
						May 10, "		2.0	Growth heavy and complete and up sides
						" 18, "		2.0	Growth heavy and complete and up sides.
						June 1, "		2.0	Growth heavy and complete and up sides.
						" 14, "		2.3	Growth mostly shaken down.
						July 1, "		0.3	Growth down completely.
						Aug. 5, "		0.3	Growth down completely.

Bouillon.	Case.	Culture.	Source.	Virulence.	Inoculation Date.	Titration. Dates.		Reaction. Per cent. Acidity.	Remarks.
Beef Inf. P1000 c.c. Peptoe 10 grams. Glycerine 40 c.c. Acidity 2 0 per cent.	Franc. B., age 24, native of Italy. Primary cervical adenitis resulting in fatal acute miliary tuberculosis.	III (Flask "b").	Pus from cervical region. Direct recovery of culture from the human tissue upon modified egg medium.	Moderately virulent for rabbits. Highly virulent for guinea-pigs. Avirulent for chickens.	February 8, 1908	Feb. 28, '08	Period under Incubator temp. R. t.	1.0	Growth heavy and blistered and covers two-thirds of surface.
						March 10, "		Neutral	Growth complete and up sides of flask.
						" 25, "		Slightly alkaline.	Growth complete and up sides of flask.
						April 15, "		0.4	Growth complete and up sides of flask.
						May 1, "		1.7	Final change in reaction.
						" 10, "		1.7	Final change in reaction.
						" 20, "		1.6	Final change in reaction.
Ditto.	Sam. B., age 20, native of Ireland. Primary cervical adenitis resulting in fatal acute miliary tuberculosis.	II (Flask "a"). "Human" biochemical reactions completely correspond to the human type of bacilli.	Necrotic material from the cervical glands. Direct isolation of culture from the human tissue upon modified egg medium.	Low degree virulence for rabbits Virulent for guinea-pigs. Avirulent for chickens.	March 24, 1908.	April 18, "	Period under Incubator temp. R. t.	1.9	Heavy growth covering three-quarters of bouillon surface.
						" 25, "		1 9	Heavy growth covering whole of bouillon surface
						May 10, "		1.5	Growth over whole surface and up sides of flask.
						" 20, "		1.0	Growth over whole surface and up sides of flask.
						June 1, "	Period under Incubator temp. Room temp.	0.7	No change in growth. Flask removed from incubator.
						" 14, "		1.4	Growth at bottom of flask. No change in reaction.
						July 4, "		4 4	Growth at bottom of flask. No change in reaction.
						Aug 6 "		4.4	Growth at bottom of flask. No change in reaction.
Ditto.	Ditto.	II (Flask "b").	Ditto	Ditto.	March 13, 1908.	April 18, "	Period under Incubator temp.	2 0	Heavy island of growth size of quarter.
						" 30, "		2.0	Heavy covering one-half of surface.
						May 10, "		2.0	Heavy growth covering three-quarters of surface.
						" 18, "		2 0	Heavy complete growth over surface.
						June 1, "		2.0	Growth heavy and thrown into deep folds.
						" 14, "		1.3	Growth heavy and up sides of flask
						July 1, "		0 9	Growth at bottom of flask for more than month
						Aug. 5, "		3.3	Growth at bottom of flask for more than two months.

Bouillon.	Case.	Culture.	Source.	Virulence.	Inoculation Date.	Titration. Dates.		Reaction. Per cent. Acidity.	Remarks.
Beef Inf. 1000 c.c. Peptone 10 grams. Glycerine 40 c.c. Acidity 2.0 per cent.	Antoine C., age 16, native of Italy. Primary cervical adenitis Recovery.	IV (Flask "a"). "Bovine" growth features and reaction upon glyc. bouillon completely correspond with "bovine bacilli."	Glands of the neck. Direct isolation upon modified egg medium.	Avirulent for rabbits. Virulent for guinea-pigs. Avirulent for chickens.	August 5, 1908.	Aug 10, '08	Period under Incubator temp.	2.0	Growth size of silver dollar.
						" 14, "		2.0	Growth covers one-half of bouillon surface.
						" 18, "		1.8	Growth completely covers surface.
						" 22, "		1.0	Growth beginning to push up sides of flask.
						" 26, "		0.5	Growth complete and up sides of flask.
						" 31, "		0.1	Growth shaken down in bottom of flask.
						Sept. 3, "		Neutral.	Growth shaken down in bottom of flask
						" 8, "		"	Growth shaken down in bottom of flask.
Ditto.	Ditto.	IV (Flask "b").	Ditto.	Ditto.	August 5, 1908.	Aug. 10, '08	Period under Inc. temp.	2.0	Growth size of half dollar.
						" 14, "		2.0	Growth covers one-half of surface.
						" 18, "		1.0	Growth covers three-quarters of surface.
						" 22, "		0.9	Growth complete
						" 26, "	Period under Room temp.	0.4	Growth complete and partly at bottom of flask.
						" 31, "		0.4	Growth complete and partly at bottom of flask.
						Sept. 3, "		0.4	Growth complete and partly at bottom of flask.
						" 8, "		0 4	Growth complete and entirely at bottom of flask.

point when grown in glycerine bouillon, nor was there any rise in acidity though the flasks were kept for months. Subsequent tests, however, have shown the fall in reaction to the neutral point to be followed by a rise in acidity. On two occasions the reaction fell slightly below the neutral point and later arose in acidity higher than the original percentage (see Table I). This latent action on glycerine occurred not only for the culture recovered directly from the human tissue but also for the one recovered through guinea-pigs. The question of a mixed tubercle infection is possible but hardly probable in this case. The glycerine broth test at first indicated that the culture was a "modified" bovine but its subsequent behavior in this medium together with other biological peculiarities make me regard it as an aberrant human type.

Culture I is the most significant example of the necessity for continued observation over a long period of time. For more than two years it always gave an atypical reaction curve in glycerine bouillon. The medium steadily increased in percentage acidity without fluctuation until it reached four or five points above the initial per cent. where it remained stationary. Recently, however, the culture has shown a complete change in its behavior upon glycerine broth; instead of the rise in percentage acidity there now occurs a gradual uninterrupted fall to 0.5 to 1.0 per cent. alkalinity from which there is no further change. This reversal of the metabolic activity of this culture under artificial cultivation is extremely interesting and would argue (1) a mixed infection in which the least saprophytic type at first predominated and subsequently the more saprophytic variety predominated, or (2) that the culture was originally an avian whose vital function had been altered during its sojourn in the animal body but not sufficiently to establish it permanently.

These "atypical" cultures in glycerine bouillon show on the whole a variation in reaction from the standard types analogous to the variation of the pneumococcus in inulin, which difference in degree of acidity corresponds with differences in growth and virulence.

Explanation of the Glycerine Bouillon Reaction.—To determine the effect of growth upon the glycerine bouillon reaction the following experiments were carried out: A series of glycerine bouillon

cultures[2] of bovine, human and avian bacilli were grown for a stated period at 37° C. All the flasks were then titrated and the reactions carefully noted. Next, one-half of these cultures (a complete series) was retained at room temperature for a further period and observations upon the reactions carried out at intervals of five and ten days. Finally, the remaining half of the cultures after titration (also a complete series) was transferred from incubator to ice-chest temperature and the reaction noted from time to time under the latter conditions. Observations extended for some series over a period of six months, others three months, and in none was it less than eight weeks. Flasks holding 500 c.c. were employed in the tests in order to contain a sufficient amount of bouillon to permit of repeated removal for titration purposes. In these tests the amount of surface growth and the reaction of the bouillon was purposely varied. The surface growth was shaken to the bottom of the flasks upon removal from the incubator. Under these conditions it was interesting to note that the human type of tubercle showed not the slightest difference in the final reaction from cultures kept undisturbed at 37° C. In flasks containing the human strains where the reaction was beginning to fall toward the neutral point at the time of removal from the incubator the acidity did not continue to lower but eventually rose to a point equal to or higher than the initial acidity (see Table I). It made not the slightest difference in the final reaction whether the growth had been shaken down from the surface of the bouillon or not. Flasks containing the bovine and avian types showed no further change in either direction after they were removed from the incubator.

The result of this test does not support the view that the difference in reaction for human and bovine mammalian types of the tubercle family depends on the ability of the living bacilli to split the glycerine molecule with the production of acids, but suggests an autolytic action on the glycerine. Again, avian strains, according to O. Bang (8) who has tested their action upon glycerine bouillon, produce only a neutral or alkaline reaction when growing

[2] Bovine IX and VIII, human XII, and avian II, which were kindly supplied me by Prof. Theobald Smith; and Cultures I, II, III and IV of my own series.

on the surface of the medium, which fact also shows that neutralization of the initial acidity is the result of growth activity.

Sometimes with a series of glycerine bouillon cultures one or more of them may fail to show the slightest change in reaction for months though the growth is complete and heavy over the whole surface and growing up the sides of the flask. At times a flask whose growth was "complete and down" for more than two months and so far had not shown the slightest change in reaction would suddenly begin to rise in per cent. acidity (see Table I).

Occasionally the change in reaction may be so long delayed that the fall to the neutral points occurs before the rise in acidity begins. Again, it is well known that with all types when grown in a broth where no glycerine has been added the reaction drops to neutral or becomes slightly alkaline and at no time shows a rise in acidity. This trend towards the neutral point must take place in glycerinated bouillon as well; however, with the human type there is in addition an action on the glycerine molecule which is distinct, and at times sufficient not only to lower but to cause a rise in the acidity of the glycerine bouillon. Since the number of bacilli undergoing disintegration varies constantly this factor in itself determines the degree of fluctuation.

That autolysis explains the rise in acidity for the human type of tubercle bacillus when grown in glycerine broth the following experiments show. A series of glycerine bouillon flasks were first titrated to show distinct variations in reaction, viz., neutral 0.5, 1.0, 2.0, and 3.0 per cent. acidity. To the separate flasks were now added five grammes of a bovine, human and avian culture which had been previously grown and removed under sterile precautions from other bouillon flasks. Each flask was now titrated to note whether any change in the reaction had occurred immediately following the addition of the culture, and then placed in the oven at 56° C. for six hours to kill the bacilli. Again after heating, the reaction was determined and the flasks placed at ice-chest temperature. Up to this date no change in reaction for any flask had occurred. Four days later the flasks still showed no change from the original reaction. About the eighth day, however, the flasks containing the killed "human" bacilli showed a decided rise in percentage acidity

which from day to day continued to advance until the final reaction was equivalent to that attained by the living glycerine-bouillon-culture under favorable conditions. The flasks containing the killed "bovine" and "avian" bacilli, respectively, failed to show at any time the slightest change in the reaction.

The fact that increased acidity occurred in the flasks containing the dead "human" bacilli and not in the "bovine" or "avian" would indicate that in the autolysis some soluble substance was liberated from the human strain that had a special action upon the glycerine molecule.

The above explanation in the rise in the acidity of glycerine cultures of human tubercle bacilli is, of course, at variance with the statement of the English Commission (11) that this phenomenon was due to the chemical interaction of continued metabolic activity on the part of the human strain.

VIRULENCE.

It is conceded by many that a considerable range in degree of virulence is possessed by the human type of the tubercle bacillus, but T. Smith (12) holds that the most virulent human cultures are not to be compared in virulence with the bovine strains. On the other hand, L. Rabinowitsch (13) states that some human cultures are more virulent for rabbits than the bovine. In this connection it is noteworthy that Culture IV of this series which is a typical bovine type of bacillus according to growth and behavior towards glycerine bouillon, has always manifested a low degree of virulence for rabbits.

Culture I when first isolated was equally virulent for rabbits and guinea-pigs and also virulent for chickens. Rabbits succumb approximately within the same period as do guinea-pigs following intravenous injection of ten milligrammes of the culture.

The view held by Arloing (14) and his pupils that bacilli from surgical tuberculosis are less virulent than those of pulmonary disease is not borne out by the study of these cultures which proves that glandular tuberculosis is not always due to bacilli of reduced virulence or to increased resistance of the host.

Again, Culture I is the exception to the rule that with tubercle

bacilli there is an inverse relation between the virulence and rapidity of growth. It grew profusely from the beginning and never showed the least tendency to lag when transferred from the solid medium to glycerine bouillon. Old cultures of the organism when kept at room temperature in sealed tubes for more than two years have remained highly virulent for guinea-pigs and rabbits; one milligramme of the culture producing extensive tuberculosis and death of the animals in four to six weeks.

These cases in some respects resemble clinically one reported by T. Smith (15) in 1898; though in his case there was an old lung focus to which he ascribed the subsequent cervical enlargement. He concluded that the rapid multiplication of the bacilli in the glands of the neck was probably due to the fortuitous assistance of other bacteria accidentally present or subsequently introduced. His culture, however, was a human type of tubercle possessing a low degree of virulence.

HISTOPATHOLOGICAL LESIONS.

Both in regard to the human and experimental lesions produced by the cultures herein reported there occurred distinct differences in the microscopic appearance, but in no way could these differences be considered as specific for any particular strain. All gradations from a subacute exudative to a proliferative type of lesion was observed.

The histopathological differences as described by Pfander (16) for fowl tuberculosis, such as (1) a relative scarcity of giant cells (2) caseation that is smooth, solid and hyaline with little tendency to calcify (3) and a tendency of the tubercle to become surrounded by a connective tissue capsule, I have observed but found too inconsistent to be of any differential value. Again, I have not been able to confirm Baumgarten's (17) view that the tubercles in fowls have less leucocytic infiltration than those in mammals. L. Rabinowitsch (3) states that in chicken tuberculosis there is a tendency for the bacilli to be diffusely disseminated and to occur in definite "heaps" which she has never observed in lesions of experimental mammalian cultures and she regards these differences as peculiar to birds, though not dependent upon any one type of tubercle bacillus. This "heaping" up of the bacilli and their spreading to all

parts of the animal body is beautifully demonstrated in Case I, which would suggest an avian infection in man.

In general the experimental lesions fall under three types: (1) the subacute exudative which is composed of polymorphonuclear leucocytes, lymphoid and plasma cells without giant or epithelioid cells; (2) one that consists entirely of epithelioid cells without necrosis or exudation, a type of lesion similar to the proliferated endothelium of the Malpighian bodies of the spleen in diphtheria and other acute infectious diseases; (3) the typical tubercular lesion consisting of epithelioid, giant cells and necrosis. All three types of lesion with intermediate stages can be produced with the same culture by grading the dosage according to the virulence and number of bacilli.

SUMMARY.

The four cultures which form the basis of this communication were recovered from peculiar cases of primary cervical adenitis in man, three of which terminated fatally of disseminated acute miliary tuberculosis in four to six weeks.

A careful comparative study shows that Culture II corresponds closely with the "human" and Culture IV with the "bovine" type of tubercle bacilli; while Cultures I and III present variations from the standard types and are to be regarded as "intermediate" or "atypical" forms.

Culture I is of unusual interest because of its remarkable variations. The clinical picture of the case, the rapid course of the infection, the enormous number of the bacilli in the tissues, their tendency to occur in "heaps" like the leprosy bacillus, the high degree of virulence alike for rabbits and guinea-pigs, the production of lesions in chickens, the ease of cultivation and the prolonged viability under unfavorable conditions, all mark the organism as a decided atypical form of tubercle bacillus in man. The prolonged viability, the production of lesions in the chicken and the great profusion of bacillary growth in the tissues would indicate an avian type. Though for years the reaction curve was atypical it has since changed completely to the "avian" curve. In this connection it is of interest to note that L. Rabinowitsch (3) states that she has iso-

lated avian tubercle bacilli from two cases of tuberculosis in man.

Cultures II and III undoubtedly belong to the human type of the tubercle family though they were under cultivation and were repeatedly tested upon glycerine broth over a period of months before their identity was definitely established.

Culture IV completely corresponds in growth and reaction in glycerine bouillon to the bovine strain; however, it manifests a low degree of virulence for rabbits which is exceptional for bovine cultures.

The old belief that bovine bacilli are more slender and beaded in the tissues and are thicker and shorter in culture than the human type, I have not been able to confirm. The morphological characters of the different cultures here reported were so inconstant that no reliance could be placed on this feature as an aid in differentiation. Outside of the animal body it would seem that the differences in size and character of the individual bacilli depend largely on the kind and reaction of the medium, whilst in the animal body they are influenced by their situation and the resistance of the host.

The nature of the growth of these tubercle cultures varies for the same culture even under apparently identical conditions. The character of the growth was never an indication of the type of the culture. It was common to obtain two distinct types of growth on the same flask of bouillon, *i. e.*, a portion of the surface would be covered with a heavy and uniformly granular layer of closely packed wax-like colonies twice the size of an ordinary pin's head, while the other portion would be a dense homogeneous layer with the typical depressed blisters.

The rapidity of growth also varied greatly for the same culture. Often in a series of twelve or more bouillon flasks which were prepared alike and inoculated with the same culture, some would cover the surface in eight days to two weeks, others would take four to six weeks, still others two to three months. It was thought in the beginning of the work that this variation might depend on the amount of oxygen within the flask or on the change in reaction in the bouillon, but further tests proved that neither of these influenced the rate of growth in any way. It would occur in loosely corked flasks as well as in those that were sealed, and in flasks where the

reaction was neutral, acid or slightly alkaline. It would seem that these changes are by no means specific for any group of the tubercle bacilli but a property possessed by them all.

The growth of the cultures on solid medium showed approximately the same variation as that from the surface of the glycerine bouillon. The wax-like colonies described by L. Rabinowitsch (3) as characteristic for avian tubercle bacilli were noted at times for all of the cultures. On the modified egg mixture the growth was always more rapid and profuse than on any other medium. I found this egg medium more certain than any other for the direct recovery of the tubercle bacillus from the tissues. Where it was desired to recover the culture from the animal tissues with certainty and celerity it had no equal. Occasionally in seven days after the inoculation of the tuberculous gland material the growth was sufficiently advanced to transplant to the bouillon flasks.

The glycerine bouillon test serves admirably to distinguish between the human, bovine and avian types of tubercle bacilli. It is also of value in the determination of degrees of adaptation in man for bacilli of the lower host-species, and in the recognition of "intermediate" types. The test to be of differential value requires repeated application and careful control over a period of months. Some freshly isolated cultures may produce their specific reaction curve in glycerine bouillon within a few weeks. On the other hand the same culture may fail to give its characteristic reaction or any alteration in the glycerine bouillon for several months though the growth has been luxuriant and complete.

The rise in acidity that occurs in glycerine bouillon for the human type of tubercle bacilli is due to a specific action on the glycerine of the products of disintegration of bacilli (autolysis); with the bovine and avian types the products of bacillary disintegration have no action on the glycerine. The fall in acidity which occurs for all three types of the tubercle bacillus is due to the products of metabolic activity.

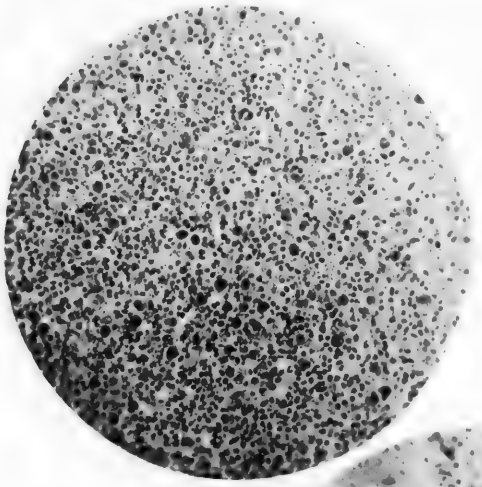


FIG. 2.

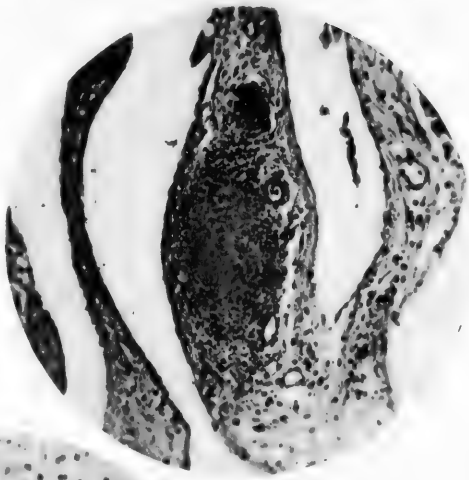


FIG. 3 (*a*).

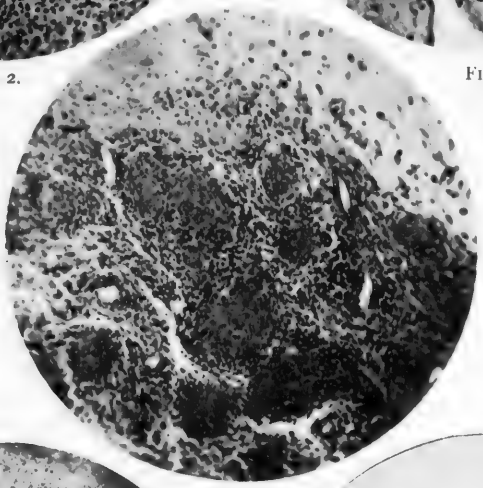


FIG. 4 (*b*).

FIG. 5 (*c*).

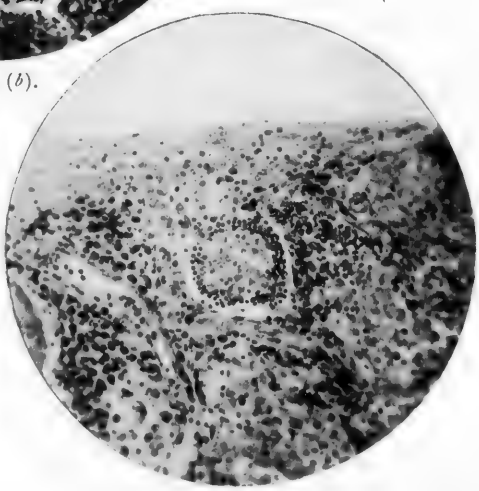


FIG. 6.

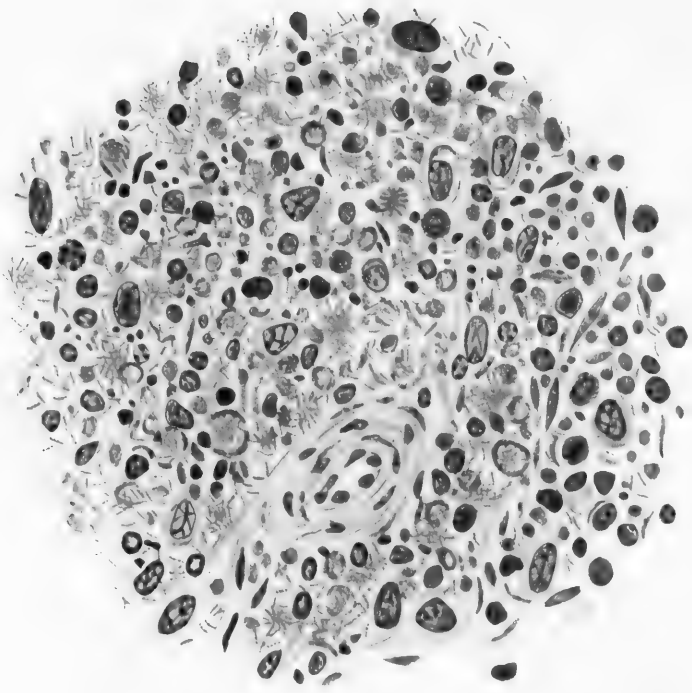


FIG. 1.

BIBLIOGRAPHY.

1. Tuberculosis Congress, London, 1901.
2. *Jour. of Med. Research,* 1905, viii, 253.
3. *Virchow's Archiv,* 1907, cxc, 246.
4. *Jour. of Exper. Med.,* 1907, ix, 352.
5. *Johns Hopkins Hospital Reports,* 1902, x, 133.
6. *Bul. of the Ayer Clin. Lab., Penn. Hospital,* 1903, 1, 4.
7. *Cent. f. Bakt., Orig.,* 1908, xlvi, 461
8. *Cent. f. Bakt., Orig.,* 1907, xliii, 34.
9. *Jour. of Med. Research,* 1905, xiii, 405.
10. *Jour. of Med. Research,* 1905, xiii, 253.
11. Royal Commission on Human and Animal Tuberculosis, Second Interim Report, 1907.
12. *Trans. of the Assoc. of Amer. Physicians,* 1896, xi, 75.
13. Arbeiten a. d. path. Inst. zu Berlin, 1906, 365.
14. Erste internat. Tuberk. Konfer. Bericht, 1902, i, 310.
15. *Jour. of the Boston Soc. of Med. Sciences,* 1898, iii, 33.
16. Arbeiten a. d. Gebiete d. path. Anat. und Bakt., 1892, i, 309.
17. Arbeiten a. d. Gebiete d. path. Anat. und Bakt., 1892, i, 322.

EXPLANATION OF PLATES XIV AND XV.

FIG. 1. Colored drawing from a section of the human spleen (Case I.) to illustrate the enormous numbers of acid-fast bacilli in the tissues. The section is stained in carbol-fuchsin, decolorized in Orth's discharging fluid and then treated with a modified Romanowski stain (Duval).

FIG. 2. Section of a lymph node of the rabbit showing the histological changes that occur after repeated intravenous injections of Culture I. Note the similarity of the cellular picture to Hodgkin's disease.

FIG. 3–5. Illustrate the lesions in the chicken following intravenous and intraperitoneal inoculations of Culture I. (*a*) Isolated tubercle in the omentum; (*b*) Multiple tubercles in the mesentery; (*c*) Large caseating nodule.

FIG. 6. Sections from the lung of the rabbit inoculated intravenously with Culture I. Note the solitary tubercle composed of epithelioid cells and surrounded with a well-defined zone of lymphoid cells.

AN EXPERIMENTAL STUDY OF THE INFLUENCE OF KIDNEY EXTRACTS AND OF THE SERUM OF ANIMALS WITH RENAL LESIONS UPON THE BLOOD PRESSURE.[1]

By RICHARD M. PEARCE, M.D.,

Professor of Pathology, The University and Bellevue Hospital Medical College, New York City.

The question of the influence of kidney extracts on the blood pressure is one which is intimately associated on the one hand with the theory of the internal secretion of the kidney, and, on the other, with the problem of the relation between cardio-vascular disturbances and renal disease. The first experimental contribution to this subject was that of Tigerstedt and Bergman (1) who offered evidence of the presence of a pressor substance in extracts of the kidney of the rabbit. This substance, which they named "renin" they regarded as an internal secretion passing normally into the blood.

Several theories, based on this principle of internal secretion, have been formulated by clinicians to explain the vascular disturbances associated with chronic nephritis. It is sufficient to mention Forlanini and Riva-Rocci (2), who hold that a blood-pressure-raising substance is formed in increased amount in the diseased kidney; and Shaw (3), who attempts to offer a basis for Traube's theory of uræmia by assuming that the substance causing arteriospasm, and thus producing cerebral disturbances without postmortem lesions, is possibly the pressor substance "renin."

Last year during the preparation of a discussion (4) of this phase of renal activity, it became necessary, on account of the confused state of the literature, to repeat these experiments, in order that the subject might be properly presented. The few experiments

[1] This investigation, aided by a grant from the Rockefeller Institute for Medical Research, was begun in the Bender Hygiene Laboratory, Albany, N. Y., and completed in the Carnegie Laboratory of The University and Bellevue Hospital Medical College, New York. Received for publication January 5, 1909.

made at that time offered no support of the theory that a pressor substance exists in the normal kidney of all animals and was therefore of little value as a basis for the consideration of the pathology of circulatory disturbances. As some of the results were, however, of physiological interest I have, during the past few months, again taken up certain phases of the general problem. The results of these investigations and also of a study of the influence on blood pressure of the serum of animals with various forms of experimental injury are here presented.

The literature is brief and may be summarized as follows:

The pressor substance of the rabbit's kidney described by Tigerstedt and Bergman in 1898 was obtained from the cortex of the kidney and was not present or was present only to a slight extent in the medulla. This substance could be extracted from the fresh organ by salt solution, alcohol, fresh blood and, to a less extent, by cold water. Extracts prepared by boiling gave no effect. The substance was non-dialysable and the investigators therefore concluded that it could not be any of the salts of the urine. To this substance they gave the name "renin" and they regarded it as an internal secretion of the kidney normally passing into the blood. The rise of pressure, which varies from a few millimeters to 25 or 35 mm. Hg, they believed to be due to an action on the peripheral nerve centers as well as possibly on the spinal cord. Very small amounts caused as much effect as larger doses and each repeated injection produced an effect as great as the first injection.

In order to demonstrate the presence of this supposed pressor substance in the circulating blood, they injected blood from the renal vein into the vessels of rabbits which had suffered double nephrectomy. A moderate rise followed. For example, in one animal, twenty-four hours after nephrectomy a rise of 18 mm. Hg occurred. These last experiments were repeated by Lewandowsky (5) the following year, and although Tigerstedt's rise of pressure was confirmed, Lewandowsky obtained a similar transient pressor effect in his controls by injecting blood from the general venous (jugular vein) and arterial systems. The rise he considered to be due in part to the rapidity of injection, but as the same rise was not obtained by injections of salt solution, he concludes that the effect

is due to some substance or substances in defibrinated blood capable of pressor effect and not to an internal secretion of the kidney.

Lewandowsky's results appear to have discouraged the further extensive investigation of Tigerstedt's observations which one would have expected. The only references in the literature, at least as far as I have been able to determine, are those of Livon (6), Fiori (7), Vincent and Sheen (8), and Shaw. Of these the first two describe a pressor effect and Vincent and Sheen observed various results, sometimes a fall, sometimes a rise in pressure, and occasionally no effect. Shaw, using the cat, obtained almost uniformly striking and prolonged rise in pressure, varying from 1 to 72 mm. Hg. Mention must also be made of the observations of Oliver (9) published a year before those of Tigerstedt. Utilizing the exposed mesentery of a frog to determine the effect of various organ extracts upon the peripheral vessels, he constantly obtained with the adrenal extract a most decisive contraction but no invariable effect with extracts of the kidney or various other organs.

When one analyzes these various observations there is some evidence to indicate that the result may depend on whether or not the kidney extract was injected into an animal of the same species. Thus Tigerstedt, in a series of about fifty experiments with the rabbit, obtained almost uniformly a pressor effect, as did also Shaw —using the cat—in all but one of nineteen injections.[2] Vincent and Sheen's injection were not always into animals of the same species and their results varied. Livon does not give the details of his work, but his results were uniform, as were also those of Fiori, who used the rabbit.

The determination of the difference between the effect of homologous and of heterologous injections has been one of the more important phases of the present investigation.

Methods.—The extracts were prepared as follows: (*a*) *Salt solution extract.* A weighed portion of kidney cortex was rubbed up in a mortar with the aid of powdered glass. Salt solution (0.85 per cent.) in the proportion of two parts to one of kidney was then added and the grinding continued. The mixture was then squeezed

[2] In a second series of experiments Shaw's results were not so uniform and he came to the conclusion that much depends on the form of anæsthesia.

through several layers of gauze and the resulting fluid filtered through paper. The fluid thus obtained was blood stained, usually opalescent, though occasionally clear; microscopically, it contained innumerable fine granules. (*b*) *Dried filtrate of alcoholic extract dissolved in salt solution.* A weighed portion of finely divided kidney cortex was rubbed up with absolute alcohol and filtered; the filtrate was evaporated to dryness at room temperature and to this was added an amount of salt solution equal to twice the bulk of the original kidney substance.

This latter extract was the one used most frequently by Tigerstedt and Bergman, presumably on account of the ease with which it could be kept without deterioration. They state, however, that the salt solution extract is of equal value, and as I found this to be true, and also as it seemed more desirable to use fresh extract, it is this extract that I have employed in the majority of my experiments. Extracts prepared by boiling and with glycerine have been used only as controls.

It must be admitted that the salt solution method of extracting is crude, but as I found, in control observations, that by its use a very active extract of the adrenal could be obtained, I have considered the method a satisfactory one.

All kidneys used in preparing extracts were obtained from animals sacrificed for that purpose, thus eliminating the influence of products formed during decomposition. The extracts were tested usually within one hour, and seldom later than two hours after obtaining the kidney substance and in the meantime were kept on ice as much as possible. The fluid in the earlier experiments was warmed before injecting to about 35° C. but as controls showed that temperature was a negligible factor, the fluid in the later work was used at room temperature. The pressure was taken in the carotid artery; the extract to be tested was injected as a rule into the saphenous vein or frequently, in the case of the rabbit, in the ear vein. All animals were anæsthetized with ether, except that in some instances rabbits were given urethane in the peritoneal cavity in doses of .025 grm. per kilo of body weight. The usual dose of kidney extract was two or three cubic centimeters, though it varied from one to five cubic centimeters in some instances.

Effect of Rabbit's Kidney Extract on the Rabbit.—Nine extracts of kidney prepared from as many rabbits were tested on nine different animals, with a total of eighteen injections. Two injections caused no change in pressure, but in the other sixteen there was a slight rise with rapid return to normal. In three of the latter a faint initial fall was observed and in two a drop followed a rise. The rise was never more than twelve and usually only 6 to 10 mm. Hg. In order to control the effect of substances in the blood, two of these extracts were made from kidneys which had been freed from blood by perfusion with salt solution. The action of these extracts did not differ from that of those made from the blood-containing kidney.

As the disturbance of pressure was very slight, a series of controls was undertaken. This series included the injection of two to three cubic centimeters of extracts of the rabbit's liver, rabbit's urine, rabbit's blood serum, Locke's solution and salt solution. Each of these was tested three times and caused almost constantly the same effect as the kidney extract. The only exceptions were absence of change in pressure in two instances when rabbit's serum and liver respectively were injected. These results appear to indicate that in an animal, as the rabbit, whose circulation is easily disturbed, the effect is largely, if not entirely, mechanical.

This conclusion is supported by further experiments in which attempts were made to remove the pressor substance. It was shown that boiled and filtered extracts, as well as the dialysate of the extract, produced the same slight transient rise of pressure as did the original kidney extract. Similar results were obtained with various alcoholic extracts, with a salt solution extract twenty-four hours old, with an extract twenty-four hours old which had been kept at a temperature of 37.5° for the last four hours of that period, and also with extracts of rabbit's kidneys which had been deprived of their blood supply by ligation for four hours.[3] Disappearance of the pressor substance was evident only in obviously putrid extracts, four or more days old; such extracts caused an immediate decided drop in pressure.

[3] These extracts caused an initial rise, which was, however, always followed by a moderate and prolonged drop in pressure.

Effect of Dog's Kidney Extract on the Dog.—This portion of the investigation has to do entirely with the study of a depressor substance of the dog's kidney. Salt solution extracts of twelve different kidneys were tested on as many dogs, with a total of twenty-nine injections. Five of the extracts caused an immediate and decided drop of 58 to 96 mm. Hg with a slow return to normal; one caused a moderate drop of 26 and one a very slight drop of 14 mm. Hg.

Three of the extracts caused a fall in pressure which ended in death. Of this I can offer no explanation; the result is apparently analogous to that observed by Pemberton and Sweet (10) after the injection into the dog of extracts of the central nervous system of the same animal.

In no instance was a rise of pressure observed, and second and third injections always produced an effect equal to the first injection. Boiled and alcoholic extracts had less effect than salt solution extracts but still possessed a very definite depressor action. It is noteworthy that the alcoholic extract was less active than the boiled extract. Only by dialysis could the depressor substance be removed; but while the dialysate contained an active depressor substance the original fluid had no pressor effect. Extracts of ligated kidneys showed no diminution in depressor action.

As the various methods of removing the depressor substance, such as those utilized by Schäfer and Vincent (11), Osborne and Vincent (12) and Joseph (13), failed to demonstrate a coexistent pressor substance, this phase of the work was abandoned.

Control injections demonstrated that dog's serum, defibrinated blood, or liver extract has no effect or a slight depressor effect while fresh urine of the dog has a decided depressor effect equal to that of the kidney extract.

These observations indicate that the depressor action of the kidney extract is due to the presence of the salts (potassium?) of the urine. This view is supported by the dialysis experiments and also by the fact that an extract of washed kidney, from which urine is presumably more or less removed, has less depressor power than an extract from the opposite unwashed kidney of the same animal.

The possibility that the depressor substance was cholin was con-

sidered, but all attempts to demonstrate cholin in the extracts by the methods described by Donath (14) were unsuccessful.

Effect of Cat's Kidney Extract on the Cat.—These experiments are few in number and were undertaken mainly for comparison with the effect on the dog of the dog's kidney extracts and of the urine. Shaw has demonstrated very conclusively the pressor effect of extracts of the cat's kidney and his results I have readily confirmed, though one of four extracts used caused a fall in pressure. The rise in pressure varies from 22 to 34 mm. Hg. The greater rise of 72 mm. Hg which Shaw describes I have not observed. This pressor effect is not a property of the cat's liver. On the other hand, the cat's urine, like that of the dog, causes a decidedly sharp drop in pressure; the lowest observed being 76 mm. Hg.

These observations therefore prove decisively that a kidney may contain a pressor substance which cannot be masked by the depressor effect of the urine, a point which could not be determined absolutely for the dog's kidney.

Heterologous Injections.—In the above experiments practically constant results have been obtained when the kidney extract has been injected into an animal of the same species as that furnishing the kidney; that is, in the rabbit a slight rise, in the cat a moderate rise and in the dog a decided fall in pressure. Cross injections made to determine the constancy of this action have given somewhat surprising results which are difficult of interpretation. An extract of the rabbit's kidney injected into the dog gives, not a rise as it does in the rabbit, but a very definite fall in pressure. This has been observed in four animals receiving four different kidney extracts. In one the drop was only 20 mm. but in the other three it was 58, 60 and 64 mm. Hg.

On the other hand, the dog's kidney extract, which in the dog produces a great fall in pressure, when injected into the rabbit causes a slight rise. This rise differs in no way from that which occurs when the rabbit receives rabbit's kidney. This has been observed in five rabbits receiving extracts of six different dog's kidneys. Likewise the dog's urine, which has a decided depressor effect on the dog, fails to act in this way on the rabbit but causes a barely perceptible transient rise of from 4 to 6 mm. Hg.

These results are difficult to explain. As far as the two animals in question are concerned each reacts to the other's kidney substance in the same way that it does to its own, even though the particular kidney substance may have had the opposite effect on the animal from which it was taken. In this way the action of kidney extracts differs from that of adrenal extracts which have a constant effect on many, if not on all animals. Whether this principle would hold with other animals is an interesting question. In this connection it is worthy of note that Brown and Joseph (15) in their study of the effect of organ extracts of cold-blooded animals on warm-blooded animals found that shark's kidney injected into the dog caused a rapid fall of 80 mm. Hg.

As the interest lay chiefly in the demonstration of pressor substances, this phase of the subject has not been further followed. The evidence is, I believe, sufficient to indicate that a pressor substance is not constantly present in all kidney extracts.

The Effect of Extracts of Kidneys with Experimental Nephritis.—The object of these experiments was to determine whether or not extracts of the kidney of experimental nephritis had an increased pressor activity. The kidneys were obtained from rabbits on the third to the fifth day of chromate or uranium poisoning, at a time when the urine contained an abundance of albumin. In no instance did such extracts have an effect different from those prepared from normal rabbit's kidney.

Effect of the Blood Serum of Animals with Lesions of the Kidneys.—If it is assumed that disease of the kidney leads to the retention of toxic products affecting blood pressure it might be possible to demonstrate these substances in the serum of animals whose kidneys had been injured in various ways. I have conducted a series of blood pressure experiments along this line. The influence of kidney reduction was first studied.

The recently published investigations of Pässler and Heineke (16) have aroused new interest in the experimental study of the relation of the kidney to increased blood pressure and cardiac hypertrophy. These investigators found that after the removal of a considerable portion of the kidney substance, approximately two-thirds to three-fourths, a rise of blood pressure occurred which

was permanent and associated with cardiac hypertrophy. This result was not constant, but occurred in about twenty-five per cent. of the animals which survived by at least four weeks a considerable reduction of kidney substance. It was observed that arterial spasm with further rise of blood pressure quickly followed stimuli which in normal animals would produce little effect. These observations suggest, according to Pässler and Heineke, that the heart hypertrophy is due to increased work resulting from the circulatory disturbances caused by tendency to arterial spasm, and that the vascular spasm is due in its turn to the effect of retained toxic substances. In the hope of demonstrating a pressor action by the serum of such animals, I have utilized the material of other investigations (17), which were carried on simultaneously with the one here described, and in which the substance of the kidney had been reduced by operation.[4]

Serum was obtained from five dogs; in four of these three-quarters of the kidney substance had been removed and the serum obtained five, thirty-four, thirty-nine, and fifty-six days respectively after the operation. The fifth lot of serum was that of a dog from which one-half of each kidney had been removed fifty-four days previously. The animals receiving the sera were under ether anæsthesia with cannula in the carotid artery; the serum was injected, in doses of twenty cubic centimeters, into the saphenous vein.

In each of the five animals thus treated a slight but definite drop in pressure, varying from 6 to 14 mm. Hg, occurred. In two animals the serum injection was repeated after several minutes had elapsed and in two others defibrinated blood was also injected. In all the same drop occurred. The injection of like quantities of

[4] Although no consistent effort was made to confirm the observations of Pässler and Heineke concerning heart hypertrophy, it is worthy of mention that in one of four dogs which lived at least five weeks after three-quarters or more of the kidney substance was removed, a definite cardiac enlargement was found. This was a dog with but one-seventh of its kidney substance remaining five weeks after the last operation. The animal gave a pressure in the carotid under ether anæsthesia of 160 and 175 mm. Hg as extremes of three observations within a half-hour period and at autopsy presented an obviously hypertrophied and slightly dilated heart. Three other animals living respectively forty-one, forty-eight and fifty-seven days showed no evidence of cardiac enlargement.

normal dog serum or of normal salt solution gave a slight rise or was followed by no appreciable change.

In like manner three dogs received the serum of animals with a spontaneous nephritis. This form of nephritis, which I have described elsewhere (18), was found in three animals during the course of this investigation. Serum was obtained from each dog and injected into another dog under the same conditions as described above. A very slight rise was obtained with one serum and an equally slight drop with the other two.

Both dogs and rabbits have been utilized in the study of the serum of experimental nephritis. A nephritis was produced by repeated subcutaneous injections of potassium chromate or uranium nitrate, and also, in the case of the rabbits, of arsenic. After three to four days, when the urine showed an abundance of albumin, the animal was bled and the effect of its serum upon the blood pressure tested on another animal of the same species. As far as I am aware the only other experiments of this type recorded are those of Ascoli and Figari (19) with nephrotoxic immune sera which I repeated several years ago with negative results (18).

The results of these experiments in the case of the dog have been rather surprising, for although each serum gives practically a uniform effect, one causes a fall and the other a rise in pressure. Thus each of four different uranium sera caused a prompt drop in pressure of from 14 to 42 mm. Hg (14, 28, 30 and 42 mm.) on initial injection and a drop of from 14 to 16 mm. on second and third injections. In no instance did this serum produce a rise or a doubtful effect. An equal number of chromate sera, on the contrary, uniformly caused a moderate but immediate rise in pressure. In one instance this was only 4 mm. Hg and represents perhaps the mere mechanical factor of injection; in the others the rise was 10 mm. in two, and 14 mm. in the fourth. A second injection in these animals caused a rise equal to the first, except in one animal which responded by a drop on the second, third and fourth injections, the drop varying from 10 to 26 mm. Hg.

Similar experiments with rabbits have given very conflicting results.[5] Chromate, uranium and arsenic sera give no uniform

[5] I wish to correct a statement in a previous (4) publication in which brief reference was made to these experiments with rabbits. It was stated that in

effect. Each may cause a rise or a fall in pressure, the fall, however, occurring three times as often as the rise. Chromate sera have given as extremes a rise of 14 and a drop of 30 mm. Hg; uranium, a rise of 38 mm. and a drop of 34 mm.; arsenic, a rise of 40 mm. and a drop of 30 mm. In all, however, the effect has been long continued, lasting for from twenty-four to forty seconds before producing maximum effect. In each instance the return to normal level has been correspondingly slow. In this respect this result with rabbits differs from the effect produced by analogous sera in the dog and from that produced in the rabbit by kidney extracts. The lack of uniformity, however, renders an interpretation impossible.

In connection with the serum injections in both dog and rabbit it may naturally be suggested that the effect is due to minute quantities of the original poison in the serum. The possibility of carrying over in the serum minute amounts of the salts injected must be considered, but it has been impossible to detect these salts in the filtrate of the serum concentrated after coagulation. The tests employed, however, are not so sensitive as entirely to exclude the persistence of these salts. On the other hand, if present, they would occur in such minute amounts that it seems improbable that they could have anything to do with the effect described. That the drop caused by uranium serum when injected into the dog is not due to traces of the uranium nitrate is shown by the fact that small amounts (0.0075 gr.) have no effect on the blood pressure, while larger amounts (0.0375 gr.) have a pressor effect. The effect of traces of chromate on the dog was unfortunately neglected. In connection with the experiments on rabbits it is noteworthy that the injection of uranium nitrate (0.0018 grm.), potassium chromate (0.0075 grm.) and arsenic (0.0025 grm.) caused a long continued fall in pressure varying from 6 to 14 mm. Hg. Although the fall is not so great as that due to the respective sera it detracts from the importance of the results with this animal.

That the effects described above are not due to substances in the normal sera or to the mechanical force of injection has been de-

rabbits both chromate and uranium sera caused a rise in pressure and never a fall. This applied to experiments made up to that time and was justified by the earlier results of the work, but the results here presented show that both sera may cause a fall in pressure more frequently than they cause a rise.

termined by injections of the serum of the respective animals and of equal amounts of salt solution. Such injections caused no change in pressure or a very slight transient rise according to the speed of injection.

Even if the experiments with rabbits are considered of doubtful value, the entire series of serum experiments, and especially those with dogs, suggests that disturbances of kidney function do cause the appearance of substances in the blood serum which have an effect on the blood pressure. Speculation concerning the nature of these bodies would be unprofitable but the variation in the dog is suggestive. Potassium chromate produces parenchymatous changes in the kidney, while uranium appears to affect both tubular and vascular structures. The difference in action of the two sera may possibly be explained by the difference in disturbance of kidney function in the animal furnishing the serum, and it is possible that more work along this line, especially with sera of chronic forms of nephritis, would yield valuable information. The presence in the serum of experimental nephritis of blood-pressure-disturbing substances is very suggestive in view of my recent study (20) of nephrotoxic substances in such serum.

SUMMARY.

1. Extracts of the rabbit's kidney injected into the rabbit cause a slight increase in blood pressure which is barely more than that due to the mechanical effect of the injection.

2. Extracts of the dog's kidney injected into the dog cause a decided fall in pressure; an equal fall may be caused by the dog's urine. A series of control experiments indicates that the fall caused by the kidney extract may be due to the urinary salts which it contains.

3. Extracts of cat's kidney cause a rise in pressure. As the cat's urine causes a fall, this rise in pressure indicates the possibility of a kidney extract containing a pressor substance which cannot be influenced by the depressor substance of the urine.

4. Rabbit's kidney, which in the rabbit produces a slight rise, when injected into the dog causes a drop comparable to that caused by the dog's kidney itself. Similarly, the dog's kidney, which in-

jected into the dog causes a drop, produces in the rabbit a rise analogous to that produced by rabbit's kidney. It is evident therefore that these pressor and depressor substances of the kidneys in question do not have a constant effect on all animals as do the extracts of the adrenal gland.

5. Extracts of kidneys which are the seat of various forms of nephritis cause the same effect as extracts of normal kidneys.

6. The serum of dogs with considerable reduction of kidney substance causes a slight fall in pressure; the serum of dogs with spontaneous nephritis gives divergent results, as does also the serum of rabbits with various forms of acute nephritis. The serum of dogs with chromate nephritis causes a slight rise, while that of dogs with uranium nephritis produces a sharp and decided fall in pressure. Although there is no uniformity in these results, their general character, and especially the experience with uranium and chromate sera of the dog, suggests that pressure-disturbing substances are present in the serum as the result of the kidney lesion. The very slight evidence of the constant presence of a pressor substance, however, offers little support to the theory that such a substance is furnished by the diseased kidney or is due to disturbances of metabolism caused by disease of the kidney.

BIBLIOGRAPHY.

1. Tigerstedt, R., and Bergman, G., Niere und Kreislauf, *Skand. Arch. f. Physiol.*, 1898, viii, 223.
2. Forlanini and Riva-Rocci, quoted from Mueller, F., Morbus Brightii, *Verhandl. d. deutsch. path. Gesellsch.*, 1905, ix, 82. Original communications cannot be traced.
3. Shaw, H. B., Autointoxication; Its Relation to Certain Disturbances of Blood Pressure, *Lancet*, 1906, i, 1295, 1375, 1455.
4. Pearce, R. M., The Theory of Chemical Correlation as Applied to the Pathology of the Kidney, *Archives of Internal Medicine*, 1908, ii, 77.
5. Lewandowsky, M., Zur Frage der inneren Secretion von Nebenniere und Niere, *Zeit., f. klin. Med.*, 1899, xxxvii, 535.
6. Livon, C., Sécrétions internes; glandes hypertensives, *Compt. rend. Soc. de biol.*, 1898, l, 98.
7. Fiori, P., Sull'azione delle iniezioni di sangue venoso emulgente e di emulsione di parenchima renale negli animali della stessa specie, *Gazz. d. ospetali*, 1904, xxv, 2019.
8. Vincent, S., and Sheen, W., The Effect of Intravascular Injections of Extracts of Animal Tissues, *Jour. of Physiol.*, 1903, xxix, 242.

9. Oliver, G., The Action of Animal Extracts on the Peripheral Vessels, *Jour. of Physiol.*, 1897, xxi, *Proc. Physiol. Soc.*, p. xxii.
10. Pemberton, R., and Sweet, J. E., The Inhibition of Pancreatic Activity by Extracts of Suprarenal and Pituitary Bodies, *Archives of Internal Medicine*, 1908, i, 628.
11. Shäfer, E. A., and Vincent, S., The Physiological Effects of Extracts of the Pituitary Body, *Jour. of Physiol.*, 1899, xxv, 87.
12. Osborne, W. A., and Vincent, S., The Physiological Effects of Extracts of Nervous Tissue, *Ibid.*, 1900, xxv, 283.
13. Joseph, D. R., Further Investigation upon the Influence of Organ Extracts of Cold-blooded Animals on the Blood Pressure, *Jour. of Exper. Med.*, 1908, ix, 606.
14. Donath, J., Das Vorkommen und die Bedeutung des Cholins . . . , nebst weiteren Beiträgen zur Chemie derselbe, *Zeit. f. physiol. Chem.*, 1903, xxxix, 526.
15. Brown, O. H., and Joseph, D. R., The Influence of Organ Extracts of Cold-blooded Animals on the Blood Pressure of Dogs, *Jour. of Physiol.*, 1906, xxxiv, 282.
16. Pässler and Heineke, Versuche zur Pathologie des Morbus Brightii, *Verhandl. d. deutsch. path. Gesellsch.*, 1905, ix, 99.
17. Pearce, R. M., The Influence of the Reduction of Kidney Substance on Nitrogenous Metabolism, *Jour. of Exper. Med.*, 1908, x, 632; also Sampson, J. A., and Pearce, R. M., A Study of the Experimental Reduction of Kidney Tissue with Special Reference to the Changes in that Remaining, *Ibid.*, 1908, x, 745.
18. Pearce, R. M., An Experimental Study of Nephrotoxins, *Univ. of Penn. Med. Bull.*, 1903, xvi, 217.
19. Ascoli and Figari, Ueber Nephrolysine, *Berl. klin. Woch.*, 1902, xxxix, 561, 634.
20. Pearce, R. M., and Sawyer, H. P., Concerning the Presence of Nephrotoxic Substances in the Serum of Animals with Experimental Nephritis, *Jour. of Med. Research*, 1908, xix, 269.

PNEUMOTHORAX AND POSTURE.[1]

By CHARLES A. ELSBERG, M.D.

(*From the Department of Physiology and Pharmacology of the Laboratories of the Rockefeller Institute for Medical Research.*)

The danger from the sudden entrance of air into the normal pleural cavity has, more than anything else, impeded the progress of the surgery of the lungs and of the other intrathoracic viscera. Because of the importance of the subject, much careful experimental and clinical work has been done, and many investigations have been made to determine the cause of the dangerous respiratory embarrassment which is apt to ensue. Little or no attention has, however, been paid to the question whether posture has any influence upon the symptoms which appear when the normal pleural cavity is laid open. In fact, this phase of the subject is mentioned in only a few publications. In a discussion on the surgery of the lungs at the congress of the French surgeons in 1906, Dépage[2] stated that he thought it best to have the patients in whom the pleural cavity had to be opened during the course of an operation flat on the abdomen. He did not, however, give any reasons why he recommended this position. Kocher,[3] in the last edition of his Operative Surgery, declares that, when the chest is to be opened, the patient should be placed on the side or abdomen, but he does not say whether he believes there is any advantage in the prone posture.

In this paper, experiments are described which were made with a view to determine what influence posture may have upon the symptoms of pneumothorax.

EXPERIMENTAL INVESTIGATIONS.

Open Pneumothorax.—The following experiments deal with the subject of pneumothorax caused by a free opening into one or both

[1] Received for publication January 8, 1909.

[2] *Congrés de chirurgie, Transactions,* 1906, xix, 387.

[3] Chirurgische Operationslehre, 1907, p. 701.

pleural cavities. The method of operation for the open pneumothorax experiments was the following:

The animal was given a hypodermic injection of morphine several hours before the experiment was begun. Under ether anæsthesia, tracheotomy was performed, a tracheal canula inserted, and the administration of ether continued through the canula. A large skin flap was then raised on one side of the chest and a subperiosteal resection of one or more ribs made. The intercostal muscles were carefully divided and the parietal pleura was exposed. While the animal was deeply under the influence of the anæsthetic, a small opening was made in the pleura and the effect noted. The opening was then gradually enlarged. In a number of the animals, the pressure of the inspired and expired air was roughly measured by allowing the animal to breathe into a large bottle, the tube leading to the bottle from the tracheal canula being connected by means of a T-tube with a water manometer. During the entire time of observation the animals were kept deeply under the influence of the anæsthetic, so that all of the reflexes were abolished.

The symptoms which ensued, in my observations, when an opening was made into one or the other pleural cavity will not be described in detail. For the purpose of this paper, the following facts will suffice. When a small opening is made into one pleural cavity of a dog, air enters the pleural cavity with a hissing noise and the lung collapses. For a moment respiration ceases, then the breathing becomes more rapid and deeper and the animal makes violent efforts at respiration. If the opening in the chest be a small one (1 to 5 mm.), one of the following several conditions will ensue: (1) After a few moments of violent respiration, expiration becomes prolonged and the heart suddenly stops beating; (2) more often the breathing will gradually return to the normal although the amount of inspired and expired air will be found to be considerably below the normal; (3) sometimes, after a few minutes, the breathing becomes irregular, violent inspiratory efforts alternate with prolonged slow expirations, the heart's action becomes slow and irregular until it ceases altogether.

Many dogs stand a small opening into the pleural cavity well. The larger the opening, however, the more likely is the occurrence

of serious interference with respiration. In almost all animals in which the size of the opening approached to or exceeded that of the diameter of the animal's trachea, dyspnea and death followed. No matter how slowly and cautiously the opening was made, typical dyspnea of a preponderant expiratory character ensued, the heart became irregular and weak, the pleural septum between the two sides bulged into the opening in the chest with each violent expiration, and rupture of the septum with double pneumothorax or sudden stoppage of the heart occurred.

The statements of most of the experimenters agree with those above given. Most of their animals died from the pneumothorax when a large opening was made in the chest. Thus Gluck lost all of the dogs he used; Biondi lost four of five; Block, Marcus, Schmidt, Pourrat and Rodet, Quenu and Longet, Sauerbruch,[4] and many others had similar experiences. It should be mentioned that in the experiments of the above named authors the animals were operated upon in the supine position.

The Influence of Posture.—(*a*) *Supine and Lateral Postures.* Openings were made in the thorax, in various places on the right and left side, near the sternum, the vertebral column, the diaphragm or the clavicle. Here again the opening was at first of small size, then it was gradually enlarged. During the observations, the posture of the animal was frequently changed, so that the animal lay sometimes on its left side, on its right side, on its back, on its abdomen; sometimes its head was raised until the animal was almost suspended vertically, sometimes it was hung up by its hind legs. In all animals, excepting those that were kept on the belly, the symptoms which ensued when a small or large opening was made in the chest were those of open pneumothorax as described above. In the experiments, no differences were found between the symptoms produced by an opening in the right as compared with one in the left pleural cavity. Twenty dogs were used in these experiments; nineteen of the animals succumbed under the typical pneumothorax symptoms when a large opening into one pleural cavity was made. One dog, however, remained alive in spite of the

[4] A complete account of the work that has been done will be found in the paper of Sauerbruch, *Mitteil. aus den Grenzgebieten der Med. und Chir.*, 1904, xiii, 399.

fact that a large opening (6 cm. in diameter) had been made in the right chest. The animal presented many of the typical symptoms of pneumothorax, but its heart action remained regular, and respiration—though irregular and weak—continued. There are some few dogs that are apparently quite insensitive to the entrance of air into the pleural cavity.

The foregoing statements may be illustrated by the following two abbreviated protocols.

EXPERIMENT 1.—Large black male. *On back;* deep anæsthesia; canula in trachea connected with large bottle and by T-tube with water manometer. Inspiration 40–50 mm., expiration 50 mm. The animal was observed for five minutes, during which time the parietal pleura was exposed in the usual manner. With a small knife an opening 4 mm. in length is made in the right pleura. Inspiration and expiration at once become violent; inspiratory pressure 4–8 mm., expiratory pressure 15–25 mm. The breathing becomes more and more labored and expiration begins to be stronger than inspiration. After three minutes, inspiration 2–5 mm., expiration 25–40 mm., the animal presents all of the symptoms of pneumothorax dyspnea. Respiration irregular, expiration prolonged, heart irregular and slow, marked bulging of mediastinal septum at each expiration. The inspiratory oscillations of the manometer are almost nothing. The heart suddenly stops beating.

EXPERIMENT 2.—Large male. *On back;* preparation as above in Experiment 1. The left pleura is exposed by resecting two ribs. Respiration 56; pulse 108. With a small knife, an opening measuring 3 mm. is now made. The animal stops breathing for 30 seconds, then breathing begins again at the same rate as before. Pulse now 136. Breathing is somewhat more labored. The animal is now turned *on its right side;* character and rate of respiration remains the same. No change is observed when the animal it turned on its *left side* or when turned *on its back* again. The opening in the pleura is now enlarged to 7 mm. Inspiration and expiration become deep and irregular; then inspiration becomes weak while expiration becomes more forcible and prolonged. With each violent expiratory effort the mediastinal septum bulges into the opening in the chest. Finally the septum ruptures and collapses, the heart then stops suddenly.

(*b*) *Prone Posture.*—In the following experiments, the animals were operated upon in the prone position, or they were first on the back and were later turned on the belly.

When a small opening is made in the pleural cavity of a dog that is lying on its belly, the animal will, in most instances, continue to breathe quietly like a normal animal. Even a very large opening (2 to 6 cm. in diameter) can be made, and breathing go on regularly and quietly, almost if not quite like the normal. Even one-

half of one chest wall can often be removed and the dog survive for hours. These statements are given after observations made in a large number of experiments, of which only the following few abbreviated protocols are given. It must be expressly stated that deep anæsthesia is essential for the success of the experiments.

EXPERIMENT 3.—Large brown bitch. *On belly;* tracheotomy; usual preparation.

4:40 p. m. Deep anæsthesia, inspiration 90 mm., expiration 70–90 mm.

4:50. Opening 3 mm. in diameter in left pleura, inspiration 40–60, expiration 50–70; animal is breathing quietly.

4:55. Opening enlarged to 10 mm., inspiration 30–40, expiration 40–50.

5:00. Opening enlarged to 2 cm., inspiration 25, expiration 30–40.

5:10. Opening enlarged to 4 cm., inspiration 20–25, expiration 30–40.

5:20. Inspiration 35–40, expiration 40–50.

5:30. Opening enlarged to 6 cm., inspiration 30, expiration 40.

5:40. Inspiration 40, expiration 40.

During the entire time of observation the animal breathed quietly and there was no evidence of dyspnea, in spite of the fact that the amount of inspired and expired air was about one-half of the normal.

EXPERIMENT 4.—Small black female. Under artificial respiration a large opening 4.5 by 7 cm. was made in the right chest while the dog was *on the belly.* Artificial respiration then stopped. The animal continued to breathe quietly and the heart action remained strong and regular for 45 minutes, when the observation ended.

EXPERIMENT 5.—Large brown male. With the animal *on the belly,* an opening 6 by 4 cm. was made in the left pleural cavity under artificial respiration. The animal continued to breathe quietly and regularly for over an hour. It was then killed.

If a dog, *on its back,* with an opening in its chest and with typical dyspnea due to the pneumothorax, is *turned on its belly,* the breathing will often become regular and quiet again, and the pressure of air breathed in and out will be found to be several times as great as when the animal was on its back. On the other hand, a dog *on its belly* with a large opening in one pleural cavity and breathing quietly, can be brought into a condition of grave dyspnea and asphyxia by *turning it on its back.* Sometimes it is even possible to resuscitate an animal that has stopped breathing by thus turning it on its belly.

This is illustrated by the following protocols:

EXPERIMENT 6.—Large black male. After the usual preparation (tracheotomy, canula connected with manometer, deep anæsthesia) the animal is placed *upon its back,* and an opening 4 mm. in diameter is made in the right

pleural cavity at 3:15 p. m. Respiration at once becomes deeper and expiratory dyspnea begins. Inspiration 90, expiration 100.

3:18 p. m. Inspiration 50, expiration 80.

3:20. Inspiration 20, expiration 90; violent dyspnea.

3:21. Opening in chest enlarged to 1 cm.; inspiration 20, expiration 90; dyspnea very violent.

3:22. Opening enlarged to 3 cm., inspiration 20, expiration 30.

3:22–½. Inspiration 5, expiration 10; heart action very irregular.

3:23. Inspiration 0, expiration 0; violent efforts at respiration, but manometer shows that no air is inhaled or exhaled. The heart suddenly stops beating. The animal is now quickly *turned on its belly* and artificial respiration given for one minute.

3:25. The animal is now breathing better again; inspiration 30 mm., expiration 40 mm., heart beating regularly.

3:26. The animal is now *turned on its back*. Dyspnea begins at once; inspiration 0–5, expiration 5–10.

3:27. *Turned on belly;* inspiration 30, expiration 40; breathing somewhat stertorous at first, but becomes gradually quiet and regular.

3:35. Animal has been breathing quietly for several minutes; it is now *turned upon its back*. Violent dyspnea begins at once, and continues until rupture of the mediastinal septum occurs two minutes later. The animal cannot be revived by artificial respiration.

Experiment 7.—Large dog. *On back;* canula in trachea connected with water manometer; deep anæsthesia; inspiration 50 mm., expiration 40–50 mm. An opening 7 mm. in diameter is made in the left pleural cavity in the usual manner. The breathing becomes slightly labored and irregular. After 12 minutes the breathing is still good although there is now well marked expiratory dyspnea. Inspiration 20–35 mm., expiration 60–90 mm.

The board on which the animal is tied is now inverted so that the dog lies with its *belly downward*. The character of the breathing improves at once and the pressure of inspired and expired air is much greater; inspiration 130, expiration 140; at times inspiration 50–70, expiration 80–120; sometimes inspiration is more deep than expiration. Breathing good though somewhat labored. The board is now turned over so that the dog lies *on its back again;* violent dyspnea begins at once. The opening in the chest is now quickly enlarged to 3 cm.; inspiration and expiration are now almost nil although the animal makes violent expiratory efforts; pulse slow and weak. The board is now quickly turned over again so that the animal hangs *belly downward;* breathing becomes better and heart action stronger. Especially noticeable is the fact that the mediastinal septum no longer bulges on expiration. A few minutes later, the board is turned again so that the dog is *again on its back;* with one violent expiratory effort the mediastinal septum ruptures and the heart stops.

A dog on its back will sometimes stand a double pneumothorax when the opening in each pleura is a very small one—not more than 1 to 2 mm. in diameter—and made very slowly. But with the dog on its belly, an opening almost 1 cm. in diameter can sometimes be made, if cautiously done, and the animal may continue to breathe

for a considerable time—sometimes for more than one hour. This was demonstrated by the following experiment:

EXPERIMENT 8.—Large brown male. *On back;* usual preparation; inspiration 140 mm., expiration 140 mm. Under deep anæsthesia; inspiration 100–140, expiration 90–100 mm. An opening 3 mm. in diameter is now made in the left pleura; inspiration 90, expiration 90. Five minutes later; inspiration 70–85, expiration 80–90; there is slight dyspnea. An opening 3 mm. in diameter is now made in the right pleura; the dog awakes from the anæsthesia and begins to struggle; inspiration 60–80 mm., expiration 100–130 mm. The animal is again deeply anesthetized; inspiration 50 mm., expiration 90 mm.; moderate expiratory dyspnea. The animal is now *turned on its belly;* inspiration and expiration remains the same, but the dyspnea is distinctly less marked. Five minutes later there is only slight expiratory dyspnea. The opening in the left pleura is now carefully enlarged to 1 cm. There is no essential change in the character of the respiration. The animal is now turned upon its back; expiratory dyspnea begins at once, heart action slow and irregular; inspiratory and expiratory pressures 0 mm. After three minutes of artificial respiration; inspiration 2–3 mm., expiration 5–15 mm. The animal is now quickly *turned upon its belly;* it is at once evident that there is much less dyspnea; inspiration 20–30 mm., expiration 30–40 mm. During the following thirty minutes the animal continues to breathe well without more than slight dyspnea.

The Relation of the Position of the Heart to the Influence of Posture.—It has been proved by the above experiments that there is a decided difference between the symptoms of pneumothorax in dogs operated upon in the prone as compared with those in the supine position. Not only did the animals present few changes in respiration and heart action when they were on the belly, if a small opening was made in the pleura, but very large openings could be made without causing serious symptoms, if the animals were kept on the belly or were turned into that position. The measurements of the pressure of inspired and expired air showed that decidedly more air was drawn in and exhaled when the animal was on its belly.

This is not due to the animal's lying on its chest, because if the animal is suspended with its belly downward (see Experiment 7) so that no pressure is made on the thorax, similar effects are to be observed. It might be due, however, to the change which occurs in the position of the heart in the thorax in the change from the supine to the prone position. When the animal lies on its back, the heart falls backward toward the vertebral column; when the animal lies upon its belly, the heart falls toward the anterior chest wall. In order to determine whether this change in the position of the heart

had anything to do with the difference in the pneumothorax symptoms, the organ was fixed to the anterior chest wall or to the vertebral column, and the effect of posture upon the symptoms in these animals was studied.

Under artificial respiration and ether anæsthesia, a small opening was made in one pleural cavity, the pericardium grasped with a forceps and brought into the wound. If the animal was operated upon in the supine position the opening in the pleural cavity was made near the sternum. In this animal, the pericardium was attached to the anterior chest wall near the sternum by a suture which passed around a costal cartilage at the sternum. If the animal was operated upon in the prone position, the pericardium was attached to the vertebral column by a suture which passed from the pericardial sac through the muscles of the back near the vertebræ. The ends of the sutures in both cases were left long so that they could be easily cut when desired. In this manner, in the animals that were operated upon while on their back, the pericardial sac and heart were pulled forward against the anterior chest wall; in the animals operated upon while on their belly, the pericardial sac and heart were pulled toward the vertebral column. After the suture had been tied, artificial respiration was stopped, and the opening in the pleural cavity enlarged. The animal was observed for a time and then the suture which fixed the heart was cut. The results of these experiments can be summed up as follows:

In the case of the dog lying *on its back* with the heart attached to the anterior chest wall so that it could not fall backwards, a large opening could be made in one pleura—almost as large as in former animals lying on the belly—without the occurrence of serious respiratory difficulty. In other words, when the heart is fixed to the anterior chest wall, a fairly large opening can be made in the pleural cavity and breathing remain good no matter what the posture of the animal. The moment, however, that the stitch was cut and the heart allowed to drop backward, the typical pneumothorax symptoms occurred.

In the case of the dog *on its belly* with the pericardial sac attached to the posterior chest wall by a suture, no more than a very small opening could be made in one pleura without the immediate appearance of pneumothorax symptoms. In other words, if the

heart is fixed to the posterior chest wall, pneumothorax symptoms appear as soon as there is an opening into the pleural cavity no matter what the posture of the animal. If the dog is lying on its belly, and the stitch is cut so that the heart can fall toward the anterior chest wall, the symptoms disappear or are much relieved, and the animal then acts as would any dog lying on its belly with an opening in one pleural cavity. Although there were some exceptions, the experiments were successful in a sufficient number of cases to justify the conclusion that the position assumed by the heart, in the prone posture, is an important factor in the difference between the symptoms produced by an opening in the pleural cavity in the prone as compared with the supine animal.

DISCUSSION AND SUMMARY.

The last mentioned fact may perhaps find its explanation in the following statements: the two pleural cavities are separated by the layers of the anterior and posterior mediastinal septa. Between the two lies the heart. In the dog, the posterior seems to be somewhat tougher than the anterior septum, and somewhat more fixed and tense. With violent respiratory movements, it is the anterior septum which more especially flaps to and fro and bulges when an opening in the pleura has been made, and it is the anterior septum which is so apt to rupture and thus cause double pneumothorax and the death of the animal. When the dog is on its back, the heart falls backward and the bulging of the anterior mediastinal septum is made more easy. It is different when the animal is on its belly. The heart falls toward the anterior chest wall and thus supports the anterior septum; hence the flapping of the septum, the interference with the respiration of the lung on the sound side, the bulging on expiration on the open side, can not so readily occur.

The danger of the open pneumothorax is greatly lessened when the animal is in the prone position. In the supine position the danger of the pneumothorax is due to the falling back of the heart and thus facilitating the rupture of the fragile anterior mediastinal septum; the danger is therefore obviated by fixing the pericardium to the anterior wall of the thorax.

I desire to state that I am greatly indebted to Dr. S. J. Meltzer for much aid and many suggestions.

THE EFFECTS OF ROENTGEN IRRADIATION UPON THE CHANGES IN THE CELL CONTENT OF THE BLOOD AND LYMPH INDUCED BY INJECTIONS OF PILOCARPINE.[1]

By ROBERT LIVINGSTON DIXON, A.B.,

Instructor of Pathology.

(*From the Pathological Laboratory of the University of Michigan.*)

The work upon which this report is based, is practically a continuation of that done by Dr. F. Peyton Rous, in this laboratory, during the last two years. Rous (1) gives a preliminary review of the experimental work done in relation to the blood changes induced by pilocarpine, and also refers to the work of Lefmann and Gasis in determining the effect of Roentgen irradiation on these changes. He also appends to his report a complete bibliography of the subject, which has been very useful in reviewing the work done under this general topic. His work had to do, particularly, with the influence of pilocarpine on the cell content of the thoracic duct as ascertained by making counts and estimations based upon the output of the duct by way of an established fistula. From his experiments he was led to state that the intravenous injection of pilocarpine nitrate causes in the dog so rapid and extensive an increase in lymphocytes in the output by way of the thoracic duct as to explain a large part, if not all, of the lymphocytosis shown in the blood of the animal under treatment with this drug. He also confirms, by calculation based upon the total amount of blood in circulation and the cell content and rate of flow of the lymph from the thoracic duct, the conclusions of previous workers (Biedl and Decastello (2), Selinoff (3), Crescenzi (4), Parodi (5)), that the thoracic duct is the principal avenue by which the lymphocytes pass from the lymphocyte-forming organs to the general blood circulation. The various theories as to how this lymphocytosis might be

[1] Received for publication January 12, 1909. Aided by a grant from the Rockefeller Institute for Medical Research.

brought about are considered critically in the body of his paper, and are left without any definite conclusion, but there is shown an inclination toward the idea of Harvey (10), *i. e.,* that the phenomenon is mainly a consequence of contraction of the smooth muscle of the spleen and lymph glands.

Before undertaking the experimentation immediately necessary to determine the point indicated by my topic, it was thought best to make a few preliminary tests in order to confirm some observations which have already been given out, also to fill out some small gaps existing in the efforts to arrive at the effects of pilocarpine on the blood elements and on the input of lymphocytes by way of the thoracic duct. The increase in white cells in the circulation after administration of pilocarpine has been so definitely established as to render it practically superfluous to repeat the observation (Horbaczewski (6), Ruzicka (7), Waldstein (8), Lefmann (9), Harvey (10), Gasis (11), Rous (1)).

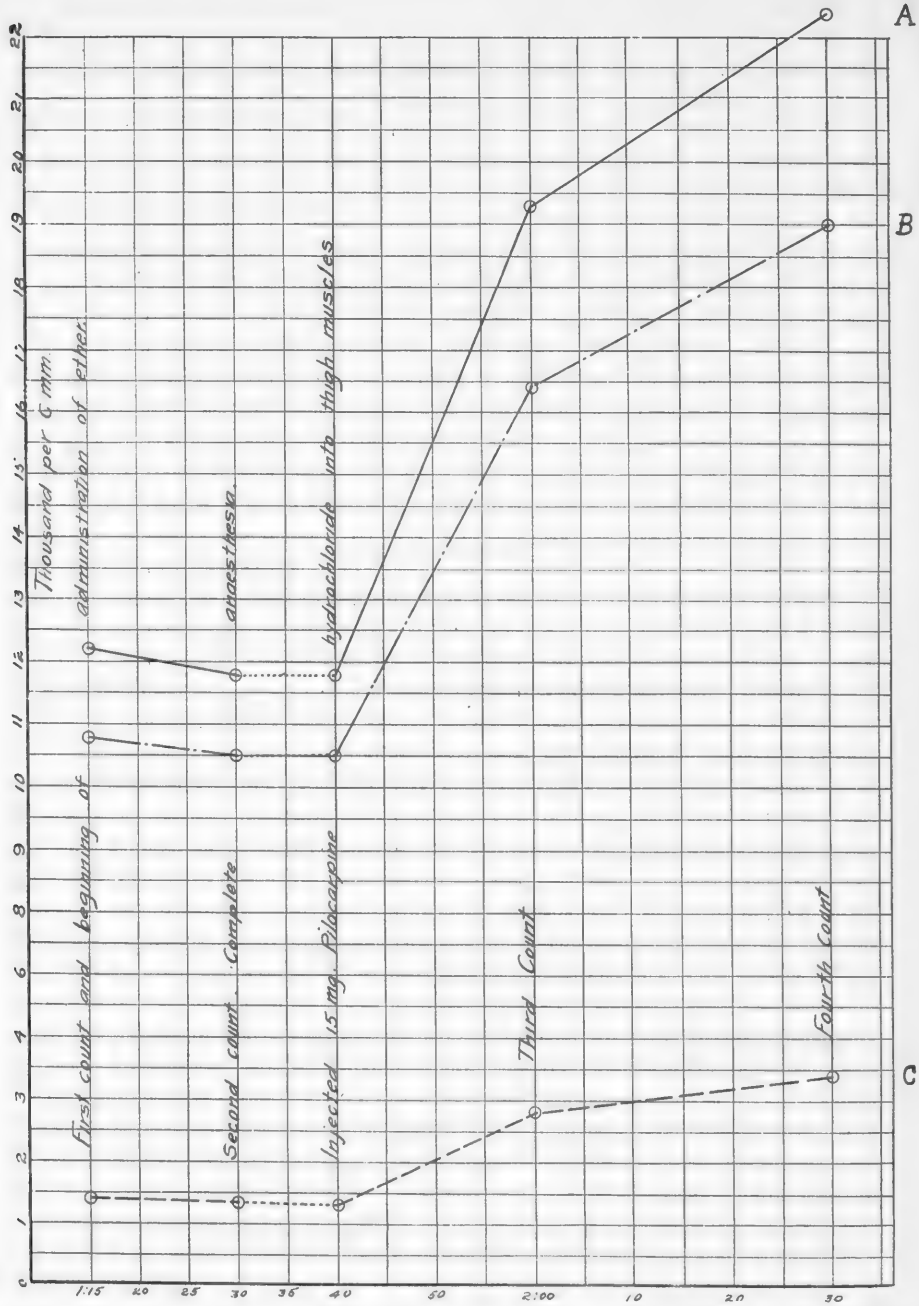
In connection with Rous's report, I may refer to an unpublished piece of work done a year ago in this laboratory by T. Dewitt Gordon. In working with pilocarpine on rabbits, he secured a great increase in white cells in the blood, characterized especially by the increase in the polymorphonuclear type of cells. As this did not seem to accord with previous tests, and especially as the rabbit can not be considered a satisfactory animal from whose blood changes any definite conclusions should be made, also because his blood counts were made with intervals of days, during which time other elements might have entered into the case so as to affect the proper changes, his work was not carried further and he made no further trials on other animals. It suggested to me, however, the fact that the " leucocytosis affecting the polymorphonuclear elements " which Rous reports as accompanying the lymphocytosis evident in his findings does not bear such a relation to the operations on the animals as to warrant attributing it at all to the surgical operations. This is clearly shown by an examination of the changes secured by Rous which I have taken the privilege to plot and describe here as Curves I, II and III.[2]

[2] In view of the detailed descriptions of Curves I, II and III these curves have been omitted.

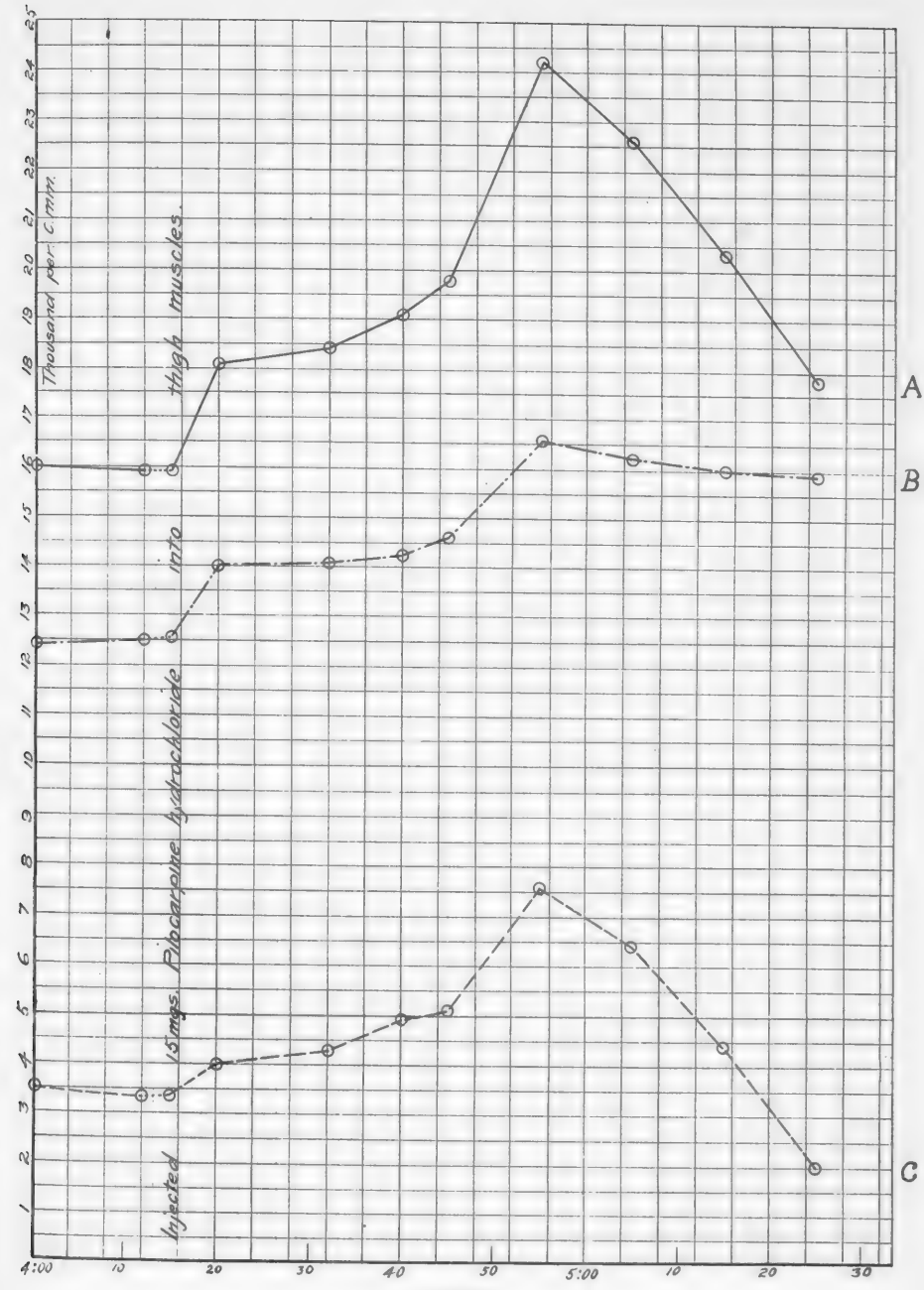
Curve I.—From this it is seen that the operation—exposure of the left external jugular vein and insertion of a cannula—was done at 9:50 o'clock. The first cell count was made at 10:15, and a second count was taken at 10:45. Now during this half hour the absolute and relative numbers of the various forms remained practically constant. The figures taken literally show a slight decrease in polymorphonuclears which, however, is so small as to be negligible. Then at 10:50 o'clock 20 mg. of pilocarpine nitrate were injected into the vein, and at the end of the next half hour the third count was made. This count showed a marked increase in number of white blood cells. Out of a total increase of 6,700 white cells per c.mm. of blood, over 5,000 constituted the increase in polymorphonuclears. Now the fact that this phenomenon was delayed until after the administration of the drug, and then appeared so promptly, seemed to me to offer reason to surmise, at least, that there might be some relation between it and the pilocarpine.

Curve Number II.—This shows the operation to have been done at 9:40, and the first count to have been made at 9:48 o'clock. A second count at 10:23 showed an increase of about 3,000 polymorphonuclears per c.mm. This, of course, had no relation to the forthcoming dose of pilocarpine which was given at 10:26 o'clock. The third count, at 11:13, gave an increase of about 1,200 mononuclears, and a further increase of over 4,000 polymorphonuclears. This gradual increase in polymorphonuclear cells from the first would appear by itself to be the consequence of the operation inasmuch as it is not materially augmented by the 10 mg. of pilocarpine.

Curve Number III.—This shows the operation to have been done at 9:30, and, unfortunately for my purpose, no count was made until the end of an hour. However, the count at the end of the hour was too small to suggest any marked increase in leucocytes which might have been instituted by the operation. A half hour later 10 mg. of pilocarpine were given intravenously, and a count made at the end of another half hour showed an increase of 6,000 white cells per c.mm. of which the increase in polymorphonuclears constituted about 5,000. Another half hour gave a further increase of only 300 mononuclear cells per c.mm., but over 2,000 polymorphonuclears per c.mm.



CURVE IV.—*A* represents total white cells per cubic millimeter of blood; *B*, polynuclear cells per cubic millimeter of blood; *C*, mononuclear cells per cubic millimeter of blood.


Thousand per C/mm
Injected 15 mgs. Pilocarpine hydrochloride into thigh muscles.
A
B
C
4:00
10
20
30
40
50
5:00
10
20
30

CURVE V.

These facts suggested to me some further experiments which I now describe briefly. First, to ascertain whether or not an operation, such as indicated above, would cause a leucocytosis so promptly and of such a degree, I exposed, after anæsthesia, an external jugular vein of a dog and inserted a cannula. A series of counts taken during the succeeding two hours showed no considerable change in total or in relative numbers of the various forms of white cells. The extreme variations were 1,200 polymorphonuclears and 250 mononuclears. This same observation was made on four other dogs subjected to other operations. There was certainly not such a leucocytosis from the operations as occurred in the animals after pilocarpine. This can be deduced from Curves VII and VIII.

The next step was to determine the effects of pilocarpine administration without any operation of consequence. The results are shown in Curves IV and V. In the first of these two cases, Curve IV, a count was made at the beginning of the anæsthetic, and another one fifteen minutes later, when anæsthesia had been established by means of chloroform followed by ether. These counts showed no considerable difference. Ten minutes later 15 mg. of pilocarpine hydrochloride were injected deep into the thigh muscles by means of an ordinary hypodermic syringe. This was followed by an immediate rise in the white blood-cell count. At the end of twenty minutes there had been an increase of 7,500 white cells per c.mm., of which the increase in polymorphonuclear cells was 6,000 per c.mm. In thirty minutes more the total increase had gone to 10,600 per c.mm. of which 8,528 were polymorphonuclear and 2,072 were mononculear. Here we had a positive augmentation of polymorphonuclear leucocytes which can not at all be attributed to any operation, but certainly is a consequence of the pilocarpine. A determination of the percentages shows that the mononuclears increased from about 11 per cent. of the total to 15 per cent.

Curve Number V.—This represents practically the same experiment, in another dog, only prolonged somewhat, and with counts made more often. Within five minutes after the injection of pilocarpine there was a sharp increase in white cells, nearly 75 per cent. of this increase being in the polymorphonuclear type. Forty

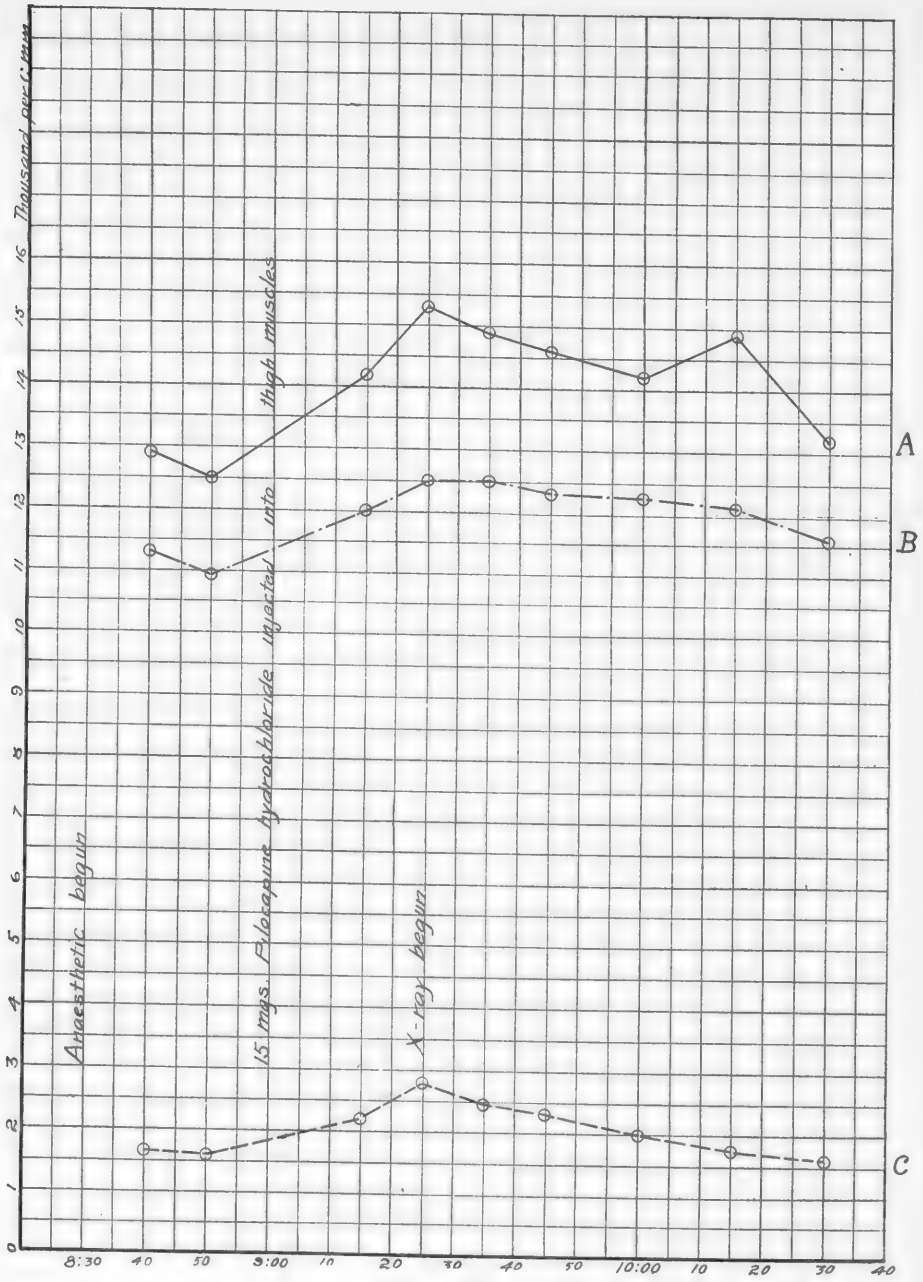
minutes after the drug was given the highest count was made. At this point the total increase consisted of 4,200 mononuclears and 4,000 polymorphonuclears per c.mm. During the succeeding thirty minutes there was a gradual decrease in the total count due almost entirely to a decrease in mononuclears. The final count showed the mononuclears to have gone 1,200 per c.mm. below the initial count, while the polymorphonuclears were still 3,200 per c.mm. above the count made at the time of beginning. This relative persistence of the polymorphonuclear increase should be given attention and will be considered further in connection with other curves showing the same feature. A determination of percentages in this case shows the mononuclears to have increased from 22 per cent. of the total to 32 per cent., and then to have gone down to 11 per cent. Such increases as are shown here, when considered on a basis of one hundred cells, would certainly attract one's attention to the lymphocytosis, as is the case in Harvey's report, but when the whole number of cells is taken into account the effect upon both types is noteworthy. The features prevailing in these experiments are also evident in the ones to be described presently, and so any conclusions which might be deduced here will be considered at the end of the next set of experiments.

The change in the lymphocyte count of the blood, produced by diverting from the blood stream the normal input by way of the thoracic duct, has been ascertained and established by several experimenters referred to above (Rous, Biedl and Decastello, Selinoff, Crescenzi, Parodi).

The following four experiments were calculated to furnish an opportunity to note the effects of Roentgen irradiation on the white blood-cells in the circulation, and on the cell content of the output from a thoracic duct fistula. The effect of the irradiation on the cell content of the circulating blood has received much attention heretofore, but, as far as I have been able to ascertain from the literature, no one has reported experiments to determine the effect on the cells in the content of the thoracic duct.

Experiment 1.—Female dog, 8 Kg., Curve VI.

The dog was anæsthetized by chloroform followed by ether. Two blood counts were made ten and tewenty minutes, respectively, later. These counts showed about 12,900 white cells per c.mm. of which 1,600 were mono-


Thousand per C.mm
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Anaesthetic begun
15 mgs. Pilocarpine hydrochloride injected into thigh muscles
X-ray begun
8:30
40
50
9:00
10
20
30
40
50
10:00
10
20
30
40
A
B
C

CURVE VI.

nuclears. At the end of a half-hour 15 mg. pilocarpine hydrochloride were injected deep into the thigh muscles of the animal. Within twenty-five minutes, the total white cell count had gone up to 15,300 per c.mm. of which 2,800 were mononuclear. At this time the animal was brought into the field of Roentgen irradiation—medium tube, eight inches from the abdomen. There began a constant decline in the number of the white cells, particularly the mononuclear type. At the end of an hour the mononuclears had been restored to the original count while the total whites were still about a thousand higher than at the beginning. This, again, shows the persistence of the leucocytosis of polymorphonuclears even in spite of the Roentgen irradiation. In this instance the interruption of the pilocarpine effect by the X-ray is evident. The picture, however, is not nearly so striking as that shown by a test reported by Lefmann (9). Starting with a lymphocyte count of about 9,000 per c.mm. he increased it by injections of pilocarpine to 17,500 per c.mm. at which time he began the Roentgen irradiation and was able to reduce the count to 3,000 per c.mm. Then he suspended the X-rays and the number of lymphocytes rapidly went up to 23,000 per c.mm. He recorded the percentage which the lymphocytes bore to the whole number of white cells. By calculation on the basis of these percentages, it can be seen that there must have been a remarkable change in the number of polymorphonuclears as well. This is shown from the following: When the lymphocytes numbered 9,000 per c.mm. their percentage was 45; when they numbered 17,500 per c.mm. their percentage was 37; and when they numbered 23,000 per c.mm. their percentage was 38. Lefmann worked with rabbits in these cases and continued the experiments over several days, which matter will be considered later.

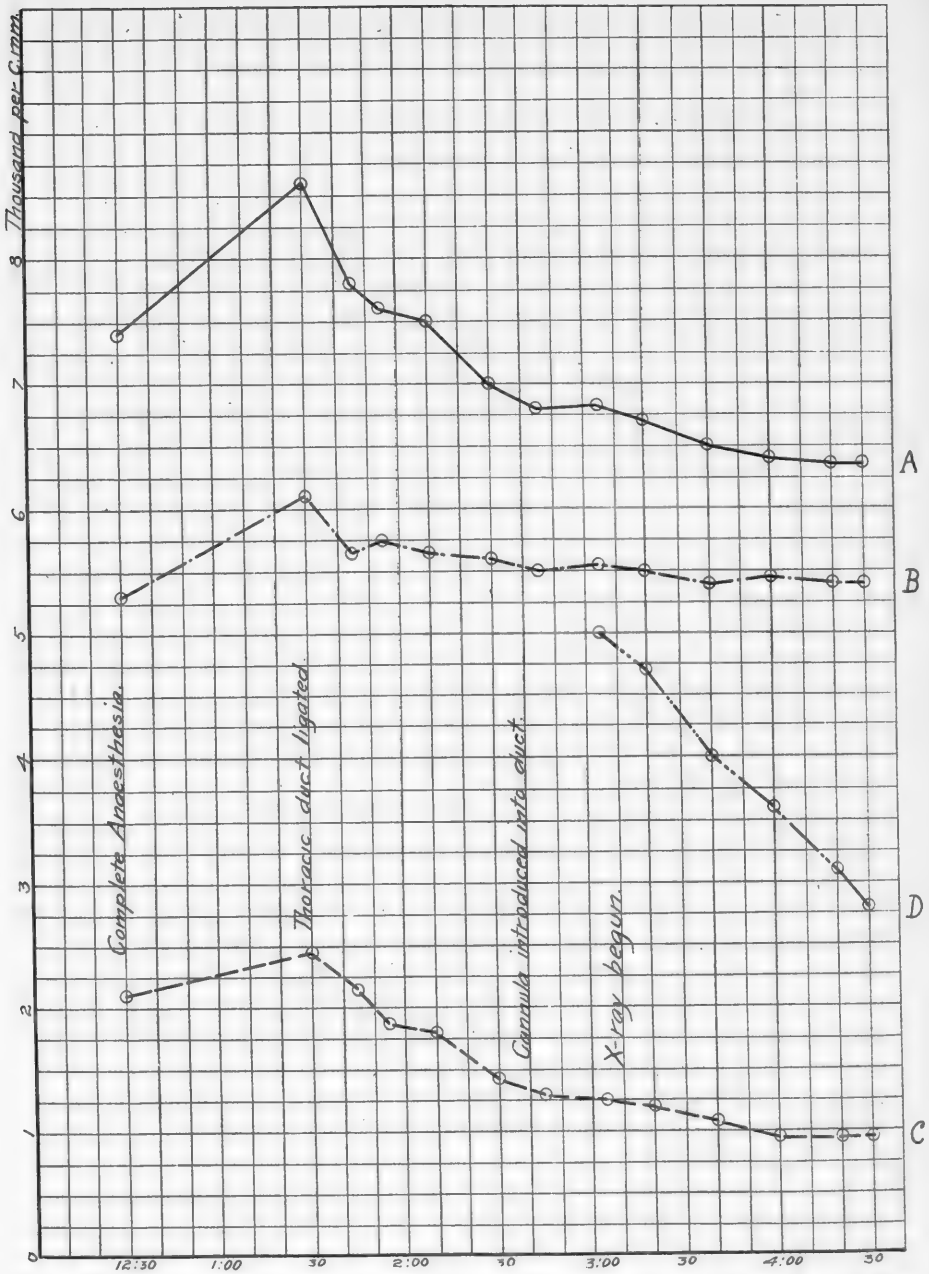
Experiment 2.—Male dog, 7Kg., Curve VII.

Anæsthesia was established by chloroform followed by ether. The initial count gave 7,400 white cells per c.mm. of blood, of which 2,100 were mononuclear. At the end of an hour the thoracic duct was ligated just before its entrance into the vein. The blood count at that time showed a rather great increase in white cells, apparently out of proportion to the operation and the time elapsed.

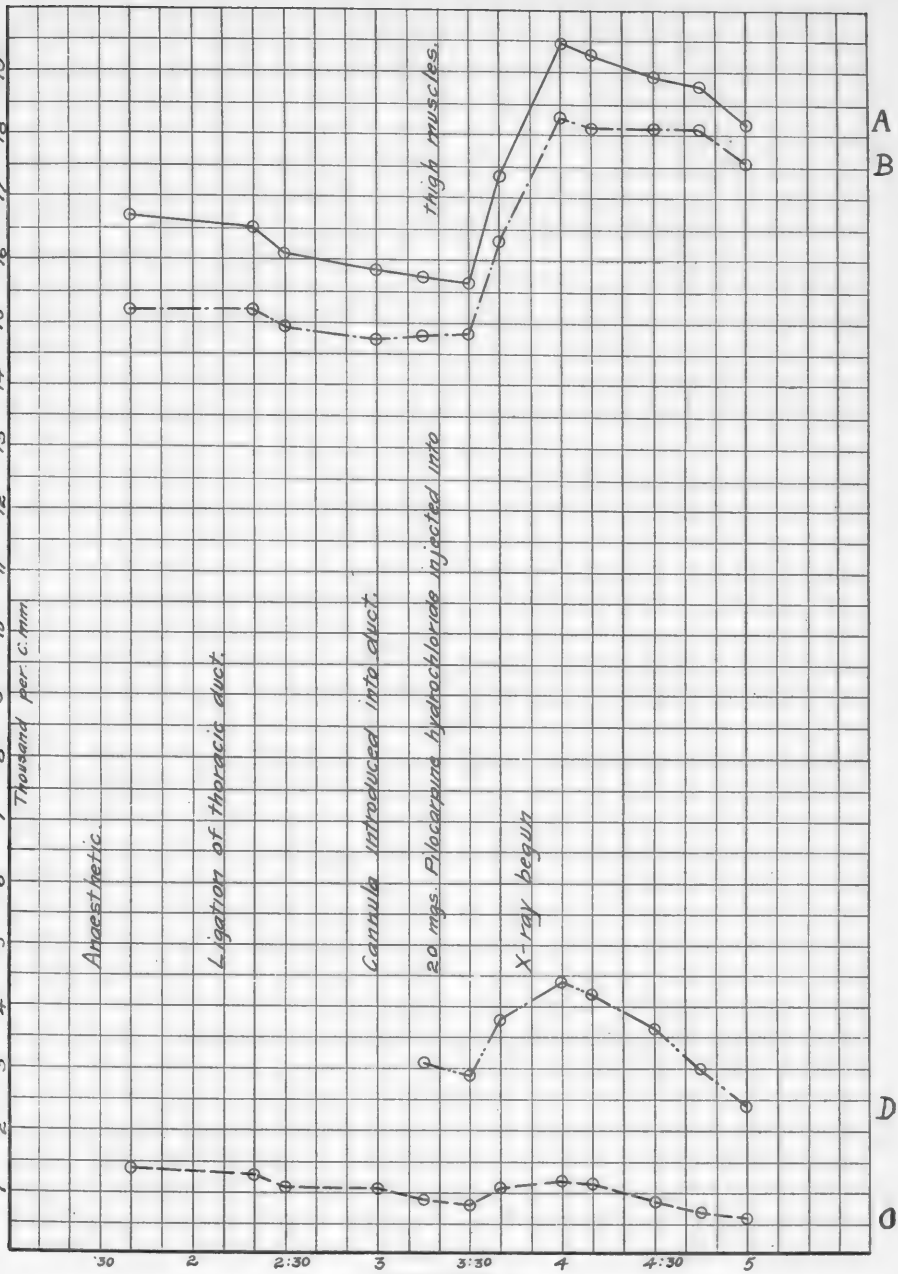
The next blood count, 15 minutes later, was near the original again, and prompts me to attribute this record of preliminary rise to error in making the count or in recording it. From then on the total whites decrease gradually, due in a great degree to a decrease in number of mononuclears. Later a cannula was introduced into the duct, and after the lymph had been allowed to flow twenty minutes, a count of the cells was made. This count gave 5,000 cells per c.mm. of lymph. Then the animal was placed in the field of Roentgen irradiation—medium tube, 8 inches from abdomen—and successive counts made of the cells in the blood, and of the cells in the outflow from the duct. In the blood the white cell number decreased generally, with some fluctuations in both types. In the duct output there occurred a definite and marked decrease in cell-content, so that at the end of an hour and a half the number of mononuclear cells had fallen from 5,000 per c.mm. to 2,800 per c.mm.

Experiment 3.—Male dog, 14 Kg., Curve VIII.

Anæsthesia was begun at 1:30 o'clock, chloroform followed by ether being used. At 1:40 the blood count showed total white cells to be 16,700 per c.mm.



CURVE VII.—*D* represents output of mononuclear cells per cubic millimeter from thoracic duct.


Thousand per c. mm
Anaesthetic.
Ligation of thoracic duct.
Canula introduced into duct.
20 mgs. Pilocarpine hydrochloride injected into thigh muscles.
X-ray begun
A
B
D
C
·30
2
2:30
3
3:30
4
4:30
5

CURVE VIII.

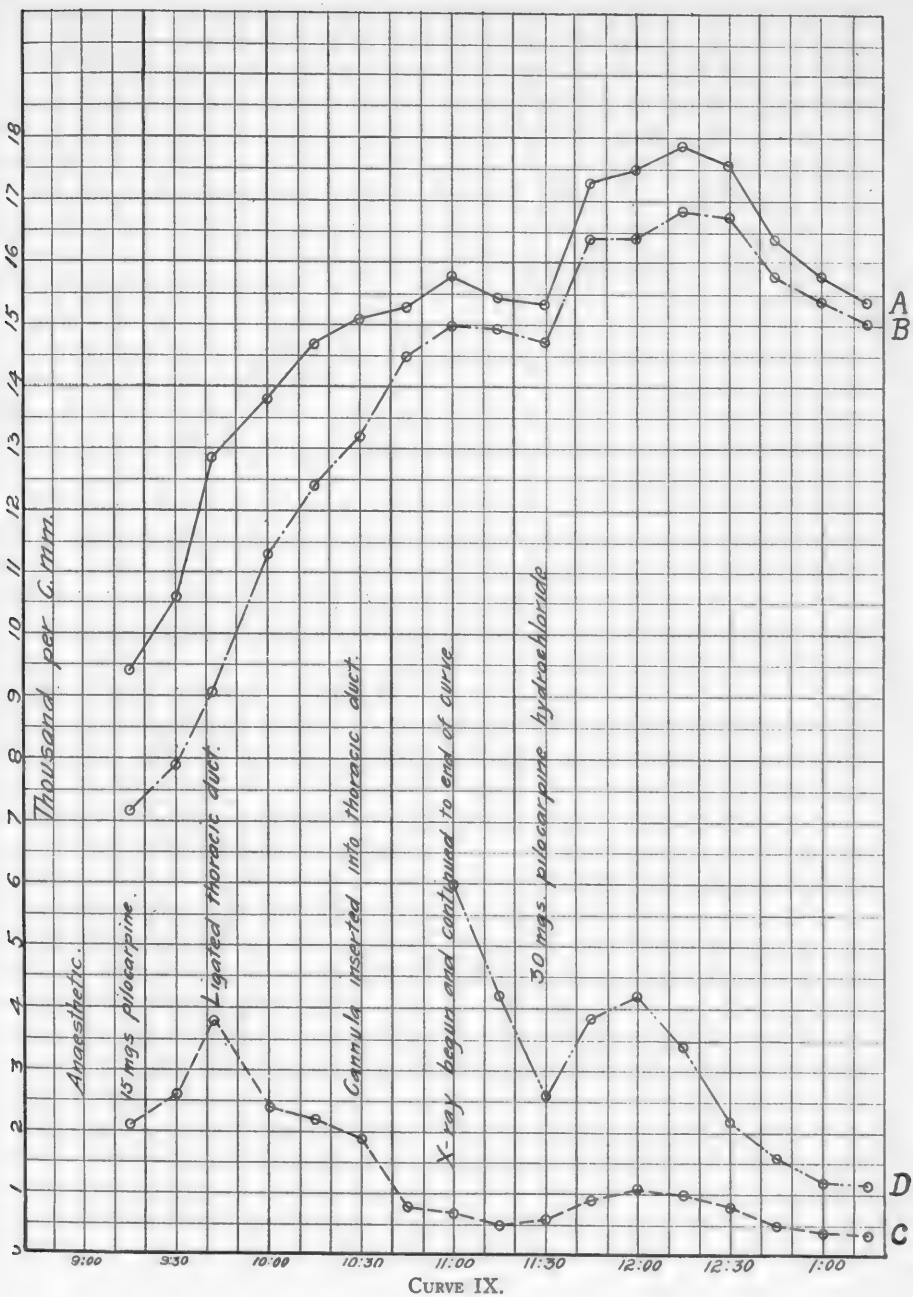
of which 1,400 were mononuclear. At 2:10 the thoracic duct was ligated, and at 3:00 a cannula had been inserted and the lymph was allowed to flow. At 3:15 o'clock the blood count showed a decrease in white cells, characterized by decrease in lymphocytes. At this time the output from the thoracic duct fistula contained 3,100 white cells. Immediately 20 mg. of pilocarpine hydrochloride were injected into the thigh muscles. The next counts at 3:30 gave still a decrease in white cells in the blood and in the duct output. At 3:40 there was an evident increase established, and the animal was exposed to Roentgen irradiation—medium tube, 8 inches from abdomen—at 3:45. The count at 4:00 o'clock gave still an evident increase of white cells in both blood and lymph, but each successive count, at intervals of about fifteen minutes, showed marked decrease in mononuclear cells in the circulation and in the lymph. At 5:00 o'clock the polymorphonuclear type remained 2,500 above the initial count, while the mononuclears were below any previous count.

This curve reaffirms the previous observations viz., (1) the increase in polymorphonuclears induced by the drug, while not so great in percentage, is remarkable in consideration of the great number of cells; (2) the persistence of the high count of polymorphonuclear cells in spite of the Roentgen rays; (3) the interruption of the lymphocytosis by Roentgen irradiation; (4) the relatively slight effect of the X-rays on the polymorphonuclear cells as compared with the effect on the mononuclear cells.

Experiment 4.—Male dog, 10 Kg., Curve IX.

Anæsthesia by chloroform followed by ether was established between 9:00 and 9:15 o'clock. Then 15 mg. of pilocarpine hydrochloride were injected deep into the thigh muscles. The white cells in the blood immediately increased. After ligation of the thoracic duct at 9:45 the mononuclear count in the blood fell, with a still increasing polymorphonuclear count. At 10:30 a cannula was inserted into the duct and the lymph allowed to flow. At 11:00 o'clock the lymph contained 6,000 mononuclear cells per c.mm., and at that time the Roentgen irradiation—medium tube, 8 inches from abdomen—was begun and continued to the end of the experiment. There followed a rapid fall in the white-cell number in the lymph, a small decrease in mononuclear cells in the circulation, and a relatively slight decrease in polymorphonuclears. I do not attribute this great fall in cell number in the lymph to be due entirely to the action of the X-rays, but in part as a consequence of the congested condition of the duct due to ligation. At 11:30, thirty milligrams of pilocarpine hydrochloride were injected. This heavy dose caused a second rise in cell counts, which, however, was soon interrupted by the continuing irradiation especially as far as concerns the mononuclear cells. At the close of the process the ploymorphonuclear cells were nearly 8,000 per c.mm. above the initial count, while the mononuclear elements were very much diminished.

This curve gives reason for especial consideration, although it does not offer any features not previously shown. In the first place the action of the drug is plainly on both types of cells, with the percentage in favor of the mononuclears. Then the effect of shutting off from the circulation the usual input by way of the duct is


Thousand per C.mm.
Anaesthetic.
15 mgs pilocarpine.
Ligated thoracic duct.
Cannula inserted into thoracic duct.
X-ray begun and continued to end of curve
30 mgs. pilocarpine hydrochloride
A
B
C
D
9:00
9:30
10:00
10:30
11:00
11:30
12:00
12:30
1:00

CURVE IX.

very striking, when we consider what the further effect of the drug would have been. Accompanying this we have the continued rise in polymorphonuclear cells, which is but slightly affected by the irradiation. Then the second and relatively large dose of pilocarpine, acting in opposition to the irradiation causes a secondary rise in all elements, which is of short duration and is succeeded by gradual and definite decline especially in the lymph.

There are some points which render it impracticable to make definite and unlimited conclusions from the foregoing sets of experiments. The variations in the thoracic duct and its tributaries render it impossible to turn completely from the circulation the great bulk of the lymph. By injecting the duct from the receptaculum upward with a colored solution various branches and irregularities become evident which could not be ascertained and taken into account in the process of establishing a thoracic duct fistula. This point might affect materially the consideration of how and where the Roentgen rays effect a diminution in lymphocytes This will be taken up in connection with my conclusions on this point.

Another factor of consequence in calculating on the count per cubic millimeter of lymph is the variation in fluid constituent. The small variations in cell count may be only relative and not absolute. This will not affect general conclusions, but might be responsible for some of the details of a conclusion. Rous made definite note of rate of flow and absolute count of cells in the lymph and showed that practically all of the lymphocytes in the general circulation might have been supplied by this source or route.

In making the total counts and also the smears for differential counts, I made use of single drops of lymph as they appeared at the end of the cannulæ, rather than making counts from several cubic centimeters, because I considered that the latter method would not give the changes in as definite relation to time and degree on account of the time necessary to collect the bulk. The matter of struggle on the part of the animal is also of consequence (Rous (1)). In these cases the anæsthesia was maintained so completely as to preclude any struggle.

The effects of the operation must be considered. Whether or

not an operation necessary to establish a thoracic duct fistula will immediately and definitely increase the white blood-cells seems to be a question. In these cases I found no satisfactory grounds for the conclusion that such is the result.

The apparently normal variations in the relative number of the various types of cells in the dog's blood, and especially the number of cells which cannot be said positively to belong to one or another type, enter into consideration materially at times.

In making the differential counts I have discarded the cells which I could not consider with a reasonable degree of certainty to belong to a certain class.

The stability of the blood of the animal used should enter into consideration. The experiments reported as done on the rabbit should be interpreted with this in view. This is especially true when the duration of the experiments is a matter of several days, and the intervals between counts are several hours. Surely it is uncertain to attribute the great changes, even, which might then appear as consequences of the drug used or of the Roentgen rays. For that reason I have done all of these experiments on dogs and used short periods of time with heavier doses of drug and irradiation.

With these possible variations in mind, I feel justified in stating some definite conclusions from the foregoing sets of experiments.

I. *Polymorphonuclear Cells.*—1. Relatively slight operations on dogs do not cause any great degree of leucocytosis within the limits of time consumed in these experiments. The substantiation of this point has been explained under the curves calculated to give the basis for the conclusion.

2. Injection of pilocarpine hydrochloride into the muscle of a dog brings about a decided increase of polymorphonuclear cells in the circulation. This is shown in every case under consideration. The increase in percentage is usually about the same as that of the mononuclear cells, but is much greater in absolute counts.

3. The polymorphonuclear leucocytosis is more persistent than the mononuclear change. This is shown in Curve V, in which case the X-ray was not used, and in Curves VIII and IX in which cases irradiation was applied.

4. The polymorphonuclear cells are but relatively slightly re-

duced in number by the action of the Roentgen rays. This is shown in Curves VI, VII, VIII, and IX.

II. *Mononuclear Cells.*—1. In the blood—(*a*) The increase in number of mononuclear cells in the blood following injection of pilocarpine hydrochloride is definite. (*b*) This increase is interrupted by the action of the X-rays and is more susceptible to their action than that of the polymorphonuclear cells. (*c*) The number is materially diminished by diverting from the blood stream the normal input by way of the thoracic duct.

2. In the output from the thoracic duct—(*a*) The number in this output is great enough, considering rate of flow and bulk of blood and lymph, to account for practically all the lymphocytes in the blood and so gives reason for the conclusion that this is the most important route by which they reach the blood. (*b*) The injection of pilocarpine hydrochloride causes a marked increase in the number entering the blood by this route (Curves VIII and IX). (*c*) This increase is interrupted by the action of Roentgen irradiation, and the number can be reduced to below the initial count (Curves VI, VII, VIII, IX).

III. *Roentgen Irradiation.*—1. On the cells in the circulating blood there is a noticeable effect. This is shown in a diminution of all forms of white cells. In each case in which the Roentgen rays were used there is shown a much greater decrease of mononuclears than of polymorphonuclears. This is perhaps due to the fact that the parent cells of the polymorphonuclear cells were not so accessible to the action of the X-rays on account of the bone tissue enclosing them, and because the long bones of the legs were practically out of the field, while the spleen and the great numbers of lymph nodes were directly in the course of the rays and not so well protected by bone.

2. The effect of the irradiation is most plainly evident on the cell content of the lymph from the thoracic duct. In each curve this is shown. From this feature of the results it is evident that the great effect of Roentgen irradiation on the mononuclear cells in the circulating blood can be explained to a great degree, at least, by its action on these cells before they reach the circulation through the thoracic duct. The fact that not all of the lymphocytes can be diverted from the bood may be made use of to explain the changes

which apparently go on in the blood under X-ray treatment after ligation of the duct. Together with this must be considered the fact that a greater body-area than the region giving supply to the thoracic duct is being irradiated, and such changes as are produced in the blood, after ligation of the duct, may be the effects of the irradiation on these accessory regions, such as the bone marrow.

It would seem then that the theory of the production of a leucotoxin in the blood, as put forward by Linser and Helber (12) and Capps and Smith (13) is not necessary to explain the destructive action of Roentgen rays upon the white cells. As has been pointed out by Rous, there is much difficulty in classifying the cells from the thoracic duct lymph. The irregularities in the cells resulting from making the smea[illegible] cause this difficulty. So in considering the effect of the pilocarpine or of the irradiation on the individual cell of the lymph, one should scarcely consider the form of the cell. From the staining reaction I could not say that the number of degenerated forms was increased after the irradiation, and should rather consider that such degenerated cells as resulted from the irradiation were retained in the blood-cell forming organs and not given into the circulation. These organs in these animals were not studied to ascertain their condition in this particular.

In reporting this investigation I am under especial obligations to Professor Warthin, who has given the work much of his attention. I also wish to thank Mr. Willey and Mr. Hill, of the Roentgenology Department, for much assistance and the use of their laboratory.

BIBLIOGRAPHY.

1. Rous, *Jour. of Exper. Med.*, 1908, x, 238, 329, 537.
2. Biedl and Decastello, *Archiv. f. d. gesam. Physiol.*, 1901, lxxxvi, 259.
3. Selinoff, *Arch. des sciences biol.*, 1903, x, 273.
4. Crescenzi, *Fol. hæmat.*, 1904, i, 418.
5. Parodi, *Arch. ital. di biolog.*, 1906, xlv, 258.
6. Horbaczewski, *Sitzungsber. der k. Akad. der Wissensch., Mathnatur wissensch. Kl.*, 1890–91, xcix–c, Abt. iii, 78.
7. Ruzicka, *Allgem. Wien. med. Zeitung*, 1893, xxxviii, 345.
8. Waldstein, *Berl. klin. Woch.*, 1895, xxxii, 368.
9. Lefmann, *Verhandl. d. Kong. f. innere Med.*, 1905, xxii, 149.
10. Harvey, *Jour. of Physiol.*, 1906, xxxv, 115.
11. Gasis, *Therap. der Gegenwart*, 1907, xlviii, 438.
12. Linser and Helber, *Verhandl. d. Kong. f. innere Med.*, 1905, xxii, 143.
13. Capps and Smith, *Trans. of the Assoc. of Amer. Physicians*, 1906, xxi, 331; *Jour. of Exper. Med.*, 1907, ix, 51.

STUDIES IN EDEMA.

II. The Influence of the Addition of Adrenalin to Solutions of Sodium Chloride and of Sodium Chloride and Calcium Chloride Solutions and of a More Rapid Rate of Inflow upon the Production of Urine, Ascites and Intestinal Fluid.[1]

By MOYER S. FLEISHER and LEO LOEB.

(From the Laboratory of Experimental Pathology of the University of Pennsylvania.)

In our first communication (1) regarding the influence of infusion of sodium chloride or mixtures of sodium chloride and calcium chloride solutions upon the production of edema, we were led to the conclusion that the addition of calcium chloride to the solutions increased the quantity of ascites and diminished the quantities of both intestinal fluid and urine. Through what action of calcium chloride these results were brought about we leave undetermined until some further experiments shall have been completed. In this paper we shall report upon a series of experiments in which we added to the sodium chloride or mixture of sodium chloride and calcium chloride solutions a certain quantity of adrenalin which is known to raise the blood pressure.

That the administration of even very small quantities of adrenalin to rabbits will raise the blood pressure is certain, and that the slow infusion of salt solution containing small quantities of adrenalin over a considerable period will produce and maintain an elevation of pressure during the entire period of infusion has been demonstrated by Kretschmer (2) in a series of experiments in which he infused dilute solutions (1:100,000 and 1:1,000,000) of adrenalin over a considerable period of time, up to three hours.

In the experiments here reported, as in our former ones, rabbits were used and our technique was in every respect the same as

[1] Received for publication January 11, 1909.

regards methods of operation, infusion and collection of the various fluids. The infused fluids were allowed to flow in at the usual rate of 4 c.c. per minute, and as in the former experiments, an 0.85 per cent. solution of sodium chloride and a 1.22 per cent. solution of calcium chloride were used; they were combined in the proportion of one volume of the latter to four volumes of the former. To these solutions adrenalin chloride (Parke, Davis and Co.) was added in a quantity of 1.5 c.c. of a 1:1,000 solution to every 1,000 c.c. of either the sodium chloride or the mixed sodium chloride and calcium chloride solutions.

NON-NEPHRECTOMIZED ANIMALS.

In a series of non-nephrectomized animals which were infused with 0.85 per cent. sodium chloride solutions to which adrenalin had been added, we found per 1,000 c.c. of fluid infused 520 c.c. of urine, and per 1,000 c.c. of fluid retained (this being the fluid infused minus the urine excreted) 79 c.c. of ascites and 58 c.c. of intestinal fluid, as compared with animals infused with pure solutions of sodium chloride (these figures are drawn from our first communication) showing 420 c.c. of urine per 1,000 c.c. of infused fluid and 68 c.c. of ascitic and 53 c.c. intestinal fluid per 1,000 c.c. of retained fluid. It may be seen that the addition of adrenalin to the salt solution has increased both urine and ascites and seemingly not affected the intestinal fluid.

TABLE I.

Non-Nephrectomized Animals Infused with 0.85 per cent. Sodium Chloride Solution Plus Adrenalin (1.5 c.c. per 1,000 c.c.).

Amount Infused.	Urine.	Ascites.	Intestinal Fluid.
600 c.c.	380 c.c.	12 c.c.	20 c.c.
800 c.c.	310 c.c.	15 c.c.	9 c.c.
800 c.c.	450 c.c.	28 c.c.	10 c.c.
800 c.c.	570 c.c.	8 c.c.	20 c.c.
800 c.c.	340 c.c.	35 c.c.	10 c.c.
800 c.c.	527 c.c.	17 c.c.	15 c.c.
800 c.c.	180 c.c.	142 c.c.	103 c.c.
800 c.c.	290 c.c.	8 c.c.	12 c.c.
800 c.c.	607 c.c.	20 c.c.	15 c.c.
800 c.c.	508 c.c.	20 c.c.	7 c.c.
800 c.c.	530 c.c.	10 c.c.	8 c.c.
800 c.c.	455 c.c.	17 c.c.	27 c.c.
800 c.c.	165 c.c.	55 c.c.	41 c.c.

Per 1,000 c.c. retained fluid: Ascites	79 c.c.
Per 1,000 c.c. retained fluid: Intestinal Fluid	58 c.c.
Per 1,000 infused fluid: Urine	520 c.c.

In a second series of non-nephrectomized animals mixtures of sodium chloride and calcium chloride solutions to which adrenalin had been added were infused, and here we find 200 c.c. of urine per 1,000 c.c. of infused fluid, and 147 c.c. of ascitic fluid and 35 c.c. of intestinal fluid per 1,000 c.c. of fluid retained. These figures may be compared with the results obtained in our earlier experiments when mixtures of sodium chloride and calcium chloride solutions were infused into non-nephrectomized animals; in these experiments we found 175 c.c. of urine per 1,000 c.c. of infused fluid, and 90 c.c. ascitic and 47 c.c. intestinal fluid per 1,000 c.c. of retained fluid.

In the new series the addition of adrenalin has not prevented the usual reduction of both urine and intestinal fluid by the calcium chloride solutions, although the reduction of urine is not so marked as when mixtures of sodium chloride and calcium chloride solutions are used alone. At the same time the ascites has been increased by the addition of adrenalin, while it seems that the intestinal fluid has been somewhat reduced.

TABLE II.

Non-Nephrectomized Animals Infused with One Volume of 1.22 per cent. Calcium Chloride Solution and Four Volumes of 0.85 per cent. Sodium Chloride Solution with the Addition of 1.5 c.c. Adrenalin per 1,000 c.c.

Amount Infused.	Urine..	Ascites.	Intestinal Fluid.
550 c.c.	36 c.c.	70 c.c.	60 c.c.
600 c.c.	44 c.c.	60 c.c.	33 c.c.
700 c.c.	58 c.c.	170 c.c.	20 c.c.
800 c.c.	77 c.c.	87 c.c.	50 c.c.
800 c.c.	180 c.c.	67 c.c.	10 c.c.
800 c.c.	115 c.c.	100 c.c.	32 c.c.
800 c.c.	318 c.c.	55 c.c.	7 c.c.
800 c.c.	280 c.c.	68 c.c.	4 c.c.
800 c.c.	60 c.c.	13 c.c.	8 c.c.
800 c.c.	287 c.c.	80 c.c.	8 c.c.
800 c.c.	330 c.c.	45 c.c.	8 c.c.
800 c.c.	189 c.c.	100 c.c.	11 c.c.

Per 1,000 c.c. infused fluid: Urine	200 c.c.
Per 1,000 c.c. retained fluid: Ascites	147 c.c.
Per 1,000 c.c. retained fluid: Intestinal Fluid	35 c.c.

NEPHRECTOMIZED ANIMALS.

In a series of nephrectomized rabbits infused with a solution of 0.85 per cent. sodium chloride to which adrenalin had been added our results show, per 1,000 c.c. of infused fluid, 119 c.c. of ascitic and 54 c.c. of intestinal fluid, as compared with 109 c.c. of ascitic and 94 c.c. of intestinal fluid in our earlier series of experiments in which pure solution of sodium chloride was injected into nephrectomized animals. The addition of adrenalin has in a similar way, as in non-nephrectomized animals, been the cause of an increase in the amount of ascites, but on the other hand it has decreased the intestinal fluid.

TABLE III.

Nephrectomized Animals Infused with 0.85 per cent. Sodium Chloride Solution Plus Adrenalin, 1.5 c.c. per 1,000 c.c.

Inflow.	Ascites.	Intestinal Fluid.
450 c.c.	33 c.c.	33 c.c.
600 c.c.	64 c.c.	26 c.c.
600 c.c.	59 c.c.	25 c.c.
600 c.c.	73 c.c.	40 c.c.
800 c.c.	123 c.c.	73 c.c.
800 c.c.	93 c.c.	100 c.c.
800 c.c.	75 c.c.	16 c.c.
800 c.c.	76 c.c.	50 c.c.
800 c.c.	100 c.c.	65 c.c.
800 c.c.	100 c.c.	105 c.c.
800 c.c.	100 c.c.	45 c.c.
800 c.c.	105 c.c.	57 c.c.
800 c.c.	98 c.c.	25 c.c.
800 c.c.	92 c.c.	40 c.c.
800 c.c.	137 c.c.	82 c.c.
800 c.c.	65 c.c.	60 c.c.
Per 1,000 c.c. infused fluid: Ascites		119 c.c.
Per 1,000 c.c. infused fluid: Intestinal Fluid		54 c.c.

We find in nephrectomized animals in which mixtures of sodium chloride and calcium chloride solutions plus adrenalin have been infused, per 1,000 c.c. of injected fluid, 165 c.c. of ascitic and 46 c.c. of intestinal fluid, as compared with 145 c.c. of ascitic and 59 c.c. of intestinal fluid in animals infused with mixtures of sodium chloride and calcium chloride solutions in our earlier experiments. The adrenalin seems to have had the same effect in these experi-

ments as when added to pure sodium chloride solutions in reducing the intestinal fluid and increasing the ascites.

TABLE IV.

Nephrectomized Animals Infused with One Volume of 1.22 per cent. Calcium Chloride Solution and Four Volumes of 0.85 per cent. Sodium Chloride Solution Plus Adrenalin, 1.5 c.c. per 1,000 c.c.

Inflow.	Ascites.	Intestinal Fluid.
360 c.c.	42 c.c.	20 c.c.
530 c.c.	65 c.c.	20 c.c.
550 c.c.	75 c.c.	10 c.c.
550 c.c.	90 c.c.	40 c.c.
600 c.c.	100 c.c.	34 c.c.
600 c.c.	90 c.c.	10 c.c.
600 c.c.	85 c.c.	47 c.c.
650 c.c.	90 c.c.	10 c.c.
650 c.c.	140 c.c.	30 c.c.
730 c.c	110 c.c.	34 c.c.
800 c.c.	135 c.c.	20 c.c.
800 c.c.	175 c.c.	72 c.c.
Per 1,000 c.c. infused fluid: Ascites		165 c.c.
Per 1,000 c.c. infused fluid: Intestinal Fluid		46 c.c.

It may be first noted that these experiments in every way confirm our former results as regards the action of the addition of calcium chloride to solutions of sodium chloride, even though in this series the addition of adrenalin has in some respects modified the results. We find that calcium chloride has reduced both the urine and the intestinal fluid and increased the ascitic fluid. We likewise note the influence of the presence or absence of the kidneys upon the ascitic and intestinal fluids, their absence causing an increase in the amount of both of these fluids.

The addition of adrenalin to the infused fluids has increased the amount of urine excreted by the rabbits about 25 per cent. when sodium chloride solutions were infused, but only about 14 per cent. when mixtures of sodium chloride and calcium chloride solutions were used. It would appear from the results of the series with non-nephrectomized animals infused with mixed sodium chloride and calcium chloride solutions that to a certain degree calcium chloride has restrained the action of adrenalin upon the excretion of urine, but that in the conflict of the influences of these two substances that

of the adrenalin has not completely disappeared. Our finding that adrenalin increases the elimination of urine is in accord with the results of Schatiloff (3), Pick and Pineles (4), and Houghton and Messil (5), who have all shown an increased diuresis following the administration of adrenalin. If, as we may assume, a constant flow of adrenalin causes a constant constriction of the small arteries, it is not possible to explain the increase in the amount of urine as being due to the increased amount of blood perfusing the capillaries of the kidney. We shall, however, leave the mechanism by which adrenalin produces diuresis undecided for the present.

The quantity of ascitic fluid has been increased by the adrenalin in every series; and when adrenalin was added to mixtures of sodium chloride and calcium chloride solutions a summation of the action of these substances takes place, this summation being most marked in the experiments with non-nephrectomized animals infused with such mixtures. In such animals the ascites has increased almost 66 per cent. over the amount noted when adrenalin had not been added to the mixtures. We see, therefore, that adrenalin increases the elimination of fluid into the peritoneal cavity.

In contradistinction to the increase of urine and ascites under the influence of adrenalin we find a constant decrease of intestinal fluid. An exception exists in the case of non-nephrectomized animals infused with sodium chloride solutions, in which the amount of intestinal fluid is approximately the same with and without the addition of adrenalin. The adrenalin certainly does not increase the intestinal fluid and in all probability it tends to diminish it.

If we now compare the actions of adrenalin and calcium chloride, we find certain resemblances and differences. They both increase the ascitic fluid and both decrease the intestinal fluid; on the other hand, whereas adrenalin increases the urine, calcium chloride causes a decrease in the amount of urine. If adrenalin and calcium chloride influence the elimination of fluid in the same direction the combination of the two substances causes an additive effect, increasing the ascites and decreasing the intestinal fluid more than if each one of these substances is used alone. When their actions are antagonistic, it appears that the influence of calcium chloride is more potent.

In our first communication we published some experiments re-

garding the influence of the rate of inflow upon the production of ascites and intestinal fluid and we used the results obtained in order to explain at least in part the difference between the amounts of intestinal and ascitic fluids in nephrectomized and non-nephrectomized animals, inasmuch as the quantity of solution infused into non-nephrectomized animals corresponds to a quantity of fluid infused at a slower rate into nephrectomized animals.

We found in a series of experiments that if fluids were infused into nephrectomized animals at a rate slower than the usual rate of four cubic centimeters per minute (namely, from two to three cubic centimeters per minute) the intestinal fluid was considerably decreased, and the ascitic fluid likewise, although to a less degree, was diminished. Nevertheless we could not exclude some other way in which extirpation of the kidneys might diminish the quantities of ascitic and intestinal fluids.

TABLE V.

Nephrectomized Animals Infused with 0.85 per cent. Sodium Chloride Solution, 8 c.c. per Minute.

Inflow.	Ascites.	Intestinal Fluid.
600 c.c.	65 c.c.	50 c.c.
600 c.c.	58 c.c.	40 c.c.
800 c.c.	57 c.c.	42 c.c.
800 c.c.	103 c.c.	108 c.c.
800 c.c.	100 c.c.	58 c.c.
800 c.c.	23 c.c.	30 c.c.
800 c.c.	90 c.c.	58 c.c.
800 c.c.	96 c.c.	110 c.c.

Per 1,000 c.c. infused fluid: Ascites 98 c.c.
Per 1,000 c.c. infused fluid: Intestinal Fluid 82 c.c.

Nephrectomized Animals Infused with One Volume of 1.22 per cent. Calcium Chloride Solution and Four Volumes of 0.85 per cent. Sodium Chloride Solution, 8 c.c. per Minute.

Inflow.	Ascites.	Intestinal Fluid.
550 c.c.	65 c.c.	20 c.c.
600 c.c.	100 c.c.	64 c.c.
640 c.c.	90 c.c.	40 c.c.
640 c.c.	120 c.c.	38 c.c.
800 c.c.	125 c.c.	50 c.c.

Per 1,000 c.c. infused fluid: Ascites 154 c.c.
Per 1,000 c.c. infused fluid: Intestinal Fluid 65 c.c.

To determine the influence of a more rapid rate of inflow we carried out a series of experiments, infusing nephrectomized animals with either 0.85 per cent. sodium chloride solutions or mixtures of one volume of 1.22 per cent. calcium chloride solution and four volumes of 0.85 per cent. sodium chloride solution at the rate of 8 c.c. per minute.

We find in animals in which sodium chloride solutions were infused, 98 c.c. ascitic and 82 c.c. intestinal fluid per 1,000 c.c. of inflow, as compared with 109 c.c. ascitic and 94 c.c. intestinal fluid at the rate of inflow of 4 c.c. per minute, and 81 c.c. ascitic and 48 c.c. intestinal fluid at from 2 to 3 c.c. inflow per minute, and 68 c.c. ascitic and 53 c.c. intestinal fluid in non-nephrectomized animals at a rate of inflow of 4 c.c. a minute. In animals into which mixed sodium chloride and calcium chloride solutions were infused at the rate of 8 c.c. per minute we find 154 c.c. ascitic and 65 c.c. intestinal fluid per 1,000 c.c. fluid infused, as compared with 145 c.c. ascitic and 59 c.c. intestinal fluid when the rate of inflow was 4 c.c. per minute; 138 c.c. ascitic and 32 c.c. intestinal fluid when the rate of inflow was 2 to 3 c.c. per minute, and 90 c.c. ascitic and 47 c.c. intestinal fluid in non-nephrectomized animals at a rate of 4 c.c. a minute.

The rapid rate of infusion has therefore increased the amount of ascites over that found with the slow inflow in both series with sodium chloride solutions, as well as with mixed sodium chloride and calcium chloride solutions. If the results of the series with rapid inflow are compared with those of the series with the usual rate of inflow, we find a slightly larger quantity of ascites in the former in the case of mixed sodium chloride and calcium chloride solutions, in the latter, in the case of pure sodium chloride solutions. The same results are noted in regard to the comparative amounts of intestinal fluid.

We wish to point out several variable factors which influence and complicate the results in these experiments. In the first place, we must take into consideration the fact that in the experiments with slow inflow relatively small quantities of fluid were infused and as we have noted before that when the figures for ascitic and intestinal fluid per 1,000 c.c. infused are calculated on the basis of experiments

in which smaller quantities of fluid were used, we obtain smaller figures for the amount of ascites. Since, therefore, the ascitic fluid increased proportionately more rapidly than did the amount of injected fluid, we cannot state how much of the diminution of ascites in the experiments with slow inflow was due to this factor (decrease of the amount of infused fluid).

On the other hand, in the experiments with rapid inflow the time during which the infused fluid acts diminishes at the same rate as the amount of fluid infused in a given time increases. These two factors may directly counteract and neutralize each other and this may explain why we do not find a larger amount of ascitic fluid with a rapid rate of inflow than with the usual rate of inflow.

SUMMARY.

1. In non-nephrectomized animals the addition of adrenalin to sodium chloride solutions or to mixtures of sodium chloride and calcium chloride solutions increases the amount of urine and of ascites, and diminishes the intestinal fluid (this diminution takes place only with mixtures of solutions of sodium chloride and calcium chloride, the quantity of intestinal fluid remaining approximately unchanged in the sodium chloride series).

2. In nephrectomized animals the addition of adrenalin to sodium chloride solutions or to mixtures of sodium chloride and calcium chloride solutions again increases the ascites and diminishes the amount of intestinal fluid in both sodium chloride and sodium chloride plus calcium chloride series.

3. We find, therefore, a summation of the action of calcium chloride and adrenalin in regard to their influence upon the formation of ascites, an antagonistic action in regard to the elimination of urine; here the influence of calcium chloride in diminishing the urine is more potent than is the action of adrenalin in increasing the amount of urine. The adrenalin decreases the elimination of fluid into the intestines and in this case we note a summation of the actions of calcium chloride and of adrenalin.

4. We see, therefore, that adrenalin and calcium chloride influence the amount of ascitic fluid and of intestinal fluid in the same direction. The amount of urine is, however, decreased by calcium chloride and increased by adrenalin.

5. If we increase the rapidity of inflow of the solutions and simultaneously decrease proportionately the time of the infusion, the amount of ascites and intestinal fluids in nephrectomized animals remains approximately unchanged, as compared with the amounts obtained at the usual rate of inflow.

BIBLIOGRAPHY.

1. Fleisher, Hoyt and Loeb, *Jour. of Exper. Med.*, 1909, xi, 291.
2. Kretschmer, *Arch. für exper. Path. u. Pharm.*, 1907, lviii, 423.
3. Schatiloff, *Arch. für Anat. u. Physiol.* (*Physiol. Abt.*), 1908, 213.
4. Pick and Pineles, *Biochem. Zeit.*, 1908, xiii, 473.
5. Houghton and Messil, *Jour. of the Amer. Med. Assoc.*, 1908, li, 1849.

STUDIES IN EDEMA.

III. The Influence of Myocarditic Lesions on the Production of Ascites, Intestinal Fluid and Urine in Animals Infused with Solutions of Sodium Chloride and of Sodium Chloride and Calcium Chloride.[1]

By MOYER S. FLEISHER and LEO LOEB.

(From the Laboratory of Experimental Pathology of the University of Pennsylvania.)

According to Lamy and Mayer (1) and Porges and Pribram (2), the infusion of calcium chloride diminishes the elimination of urine through its influence on the circulatory system; by lowering the blood pressure (Porges and Pribram) or by constriction of the renal vessels and diminution of the amount of blood passing through the kidneys (Lamy and Mayer). We have found in previous investigations (3) that calcium chloride diminishes the urine and intestinal fluid and has the reverse effect upon the transudation into the peritoneal cavity. In order to determine how much of the action of calcium chloride might be due to the lowering of the blood pressure, how much to a direct chemical action on the cells lining the cavities into which the fluid is eliminated, we searched for a method which would in all likelihood lower the blood pressure without exerting any chemical or physico-chemical action on these cells. As we shall describe more in detail in another paper, we found a simple method of produing chronic interstitial myocarditis in rabbits.

By a single injection of adrenalin and either sparteine sulphate or sodium caffeine benzoate we were able to produce such lesions in more than 60 per cent. of rabbits used in these experiments. This lesion appeared in the left ventricle after the injection and consisted mainly in an overgrowth of interstitial tissue, hypertrophy of the muscle fibers and some degenerative changes in the muscle cells.

We desired to test the action of such hearts under conditions in

[1] Received for publication January 11, 1909.

which an increased amount of work was required, namely, after infusion of large amounts of fluid into the circulation. We assumed that such hearts under these conditions would prove to be relatively inferior in power to normal hearts and that possibly a lowering of the blood pressure would follow if such animals were infused with sodium chloride solutions and mixtures of sodium chloride and calcium chloride solutions.

The technique in these experiments was in all particulars the same as that described in our first communication and the solutions infused were either an 0.85 per cent. sodium chloride solution or a mixture of four volumes of an 0.85 per cent. sodium chloride solution and one volume of 1.22 per cent. calcium chloride solution.

As stated above, in about 60 per cent. of the animals which had been injected for the purpose of producing the myocarditic lesion, the change in the heart was marked enough to be seen with the naked eye; in an additional number of animals a slight lesion was present which became apparent only on microscopic examination, and in a certain number of cases no lesion was present even on microscopic examination. In the following report of our experiments we shall divide the animals into two groups—one in which a gross lesion was present, and a second in which the heart appeared normal to the naked eye.

EXPERIMENTS WITH NON-NEPHRECTOMIZED ANIMALS.

In one series of animals which showed a heart lesion following the above mentioned injection of adrenalin, we infused sodium chloride solution and found in this series an average of 80 c.c. of urine per 1,000 c.c. of fluid infused, 71 c.c. of ascitic and 55 c.c. of intestinal fluid per 1,000 c.c. of retained fluid (by retained fluid we understand infused fluid minus the amount of urine), as compared with 420 c.c. of urine per 1,000 c.c. of infused fluid and 68 c.c. ascitic and 53 c.c. intestinal fluid per 1,000 c.c. of retained fluid in non-nephrectomized animals which had not been injected with the sparteine and adrenalin, and in which the heart was therefore normal. In the second series of animals which had received injections of sparteine and adrenalin but which did not show any gross cardiac lesions we found 420 c.c. of urine per 1,000 c.c. of infused fluid,

65 c.c. of ascitic and 40 c.c. of intestinal fluid per 1,000 c.c. of retained fluid.

TABLE I.

Non-Nephrectomized Rabbits Showing Gross Cardiac Lesions Infused with 0.85 per cent. Sodium Chloride Solution.

Inflow.	Urine.	Ascites.	Intestinal Fluid.
200 c.c.	14 c.c.	8 c.c.	7 c.c.
300 c.c.	7 c.c.	46 c.c.	10 c.c.
320 c.c.	5 c.c.	15 c.c.	13 c.c.
460 c.c.	9 c.c.	36 c.c.	24 c.c.
560 c.c.	20 c.c.	20 c.c.	70 c.c.
560 c.c.	42 c.c.	37 c.c.	32 c.c.
600 c.c.	17 c.c.	60 c.c.	53 c.c.
650 c.c.	35 c.c.	42 c.c.	20 c.c.
800 c.c.	100 c.c.	60 c.c.	25 c.c.
800 c.c.	175 c.c.	20 c.c.	5 c.c.

Per 1,000 c.c. infused fluid: Urine 80 c.c.
Per 1,000 c.c. retained fluid: Ascites 71 c.c.
Per 1,000 c.c. retained fluid: Intestinal Fluid 55 c.c.

Non-Nephrectomized Rabbits Showing no Gross Cardiac Lesion Infused with 0.85 per cent. Sodium Chloride Solution.

Inflow.	Urine.	Ascites.	Intestinal Fluid.
600 c.c.	230 c.c.	22 c.c.	10 c.c.
700 c.c.	450 c.c.	34 c.c.	25 c.c.
800 c.c.	370 c.c.	42 c.c.	5 c.c.
800 c.c.	340 c.c.	35 c.c.	10 c.c.
800 c.c.	170 c.c.	8 c.c.	36 c.c.

Per 1,000 c.c. infused fluid: Urine 420 c.c.
Per 1,000 c.c. retained fluid: Ascites 65 c.c.
Per 1,000 c.c. retained fluid: Intestinal fluid 40 c.c.

In the series of rabbits which have shown a heart lesion and have been infused with mixtures of sodium chloride and calcium chloride solutions we find 125 c.c. of urine per 1,000 c.c. of infused fluid, 125 c.c. of ascitic and 22 c.c. of intestinal fluid per 1,000 c.c. of retained fluid. In normal animals infused with such mixtures we find 175 c.c. of urine per 1,000 c.c. infused fluid, and 90 c.c. ascitic and 47 c.c. intestinal fluid per 1,000 c.c. retained fluid. In a few (three) animals which have shown no gross heart lesion we find 148 c.c. urine per 1,000 c.c. infused fluid, 174 c.c. ascitic and 38 c.c. intestinal fluid per 1,000 c.c. retained fluid. As we have shown

before we cannot draw any definite conclusion from a series of three experiments and we mention these latter figures without regarding them as conclusive.

TABLE II.

Non-Nephrectomized Rabbits Showing Gross Cardiac Lesions Infused with One Volume 1.22 per cent. Calcium Chloride and Four Volumes 0.85 per cent. Sodium Chloride Solutions.

Inflow.	Urine.	Ascites.	Intestinal Fluid.
470 c.c.	20 c.c.	70 c.c.	35 c.c.
530 c.c.	43 c.c.	65 c.c.	10 c.c.
640 c.c.	66 c.c.	115 c.c.	5 c.c.
700 c.c.	22 c.c.	42 c.c.	5 c.c.
700 c.c.	255 c.c.	65 c.c.	5 c.c.
800 c.c.	80 c.c.	64 c.c.	15 c.c.

Per 1,000 c.c. infused fluid: Urine	125 c.c.
Per 1,000 c.c. retained fluid: Ascites	125 c.c.
Per 1,000 c.c. retained fluid: Intestinal Fluid	22 c.c.

Non-Nephrectomized Rabbits Showing no Gross Cardiac Lesions Infused with One Volume 1.22 per cent. Calcium Chloride and Four Volumes 0.85 per cent. Sodium Chloride Solutions.

Inflow.	Urine.	Ascites.	Intestinal Fluid.
500 c.c.	70 c.c.	77 c.c.	20 c.c.
600 c.c.	100 c.c.	80 c.c.	10 c.c.
760 c.c.	104 c.c.	120 c.c.	30 c.c.

Per 1,000 c.c. infused fluid: Urine	148 c.c.
Per 1,000 c.c. retained fluid: Ascites	174 c.c.
Per 1,000 c.c. retained fluid: Intestinal Fluid	38 c.c.

EXPERIMENTS WITH NEPHRECTOMIZED ANIMALS.

We found in a series of experiments with animals showing gross cardiac lesions from which both kidneys had been removed before the infusion with sodium chloride solution 76 c.c. of ascitic fluid and 96 c.c. of intestinal fluid per 1,000 c.c. of fluid infused as compared with 109 c.c. ascitic and 94 c.c. intestinal fluid per 1,000 c.c. of infused fluid, as found in normal nephrectomized animals (3). In a later series of ten experiments with nephrectomized normal animals which were not included in our first communication, we obtained 87 c.c. ascitic and 76 c.c. intestinal fluid per 1,000 c.c. of infused fluid. In a similar way we found last year in a series carried on during the winter months a smaller amount of ascites than

in a series carried on during the summer; we found, however, at the same time in control experiments that such variations did not affect our main conclusions. In animals which had been injected with adrenalin and sparteine, etc., but which at autopsy showed no macroscopic cardiac lesion, 79 c.c. of ascitic and 80 c.c. of intestinal fluid were present.

A series of experiments in which nephrectomized animals with cardiac lesions were infused with mixed sodium chloride and calcium chloride solutions was omitted, as it seemed that the experiments already enumerated showed quite distinctly the effect of the cardiac lesion.

TABLE III.

Nephrectomized Rabbits Showing Gross Cardiac Lesions Infused with 0.85 per cent. Sodium Chloride Solutions.

Inflow.	Ascites.	Intestinal Fluid.
560 c.c.	20 c.c.	70 c.c.
600 c.c.	80 c.c.	80 c.c.
600 c.c.	48 c.c.	32 c.c.
650 c.c.	35 c.c.	51 c.c.
680 c.c.	45 c.c.	85 c.c.
700 c.c.	25 c.c.	100 c.c.
800 c.c.	70 c.c.	27 c.c.
800 c.c.	85 c.c.	95 c.c.
800 c.c.	45 c.c.	65 c.c.
800 c.c.	60 c.c.	90 c.c.

Per 1,000 c.c. infused fluid: Ascites 76 c.c.
Per 1,000 c.c. infused fluid: Intestinal Fluid 98 c.c.

Nephrectomized Rabbits Showing Gross Cardiac Lesions Infused with Sodium Chloride Solutions.

Inflow.	Ascites.	Intestinal Fluid.
400 c.c.	30 c.c.	45 c.c.
430 c.c.	35 c.c.	35 c.c.
600 c.c.	10 c.c.	100 c.c.
650 c.c.	80 c.c.	30 c.c.
650 c.c.	40 c.c.	55 c.c.
800 c.c.	40 c.c.	60 c.c.
800 c.c.	54 c.c.	30 c.c.
800 c.c.	130 c.c.	75 c.c.
800 c.c.	30 c.c.	70 c.c.
800 c.c.	60 c.c.	65 c.c.

Per 1,000 c.c. infused fluid: Ascites 79 c.c.
Per 1,000 c.c. infused fluid: Intestinal Fluid 80 c.c.

Considering first the influence of experimental myocarditic lesions upon the excretion of urine, it is quite evident that such lesions cause a marked decrease in the amount of urine; this is especially marked, if solutions of sodium chloride are infused, the urine being reduced to less than one-fifth of the usual quantity; if calcium chloride has been added to the infused fluid, the urine is only reduced to about five-sevenths of the quantity secreted in normal animals infused with mixtures of sodium chloride and calcium chloride solutions. The reduction of urine is therefore relatively much more marked in animals infused with sodium chloride solutions than in those infused with mixed sodium chloride and calcium chloride solutions. That this marked reduction of urine is due to a renal lesion which might arise from the same cause as the cardiac lesion or possibly be secondary can be excluded, because we were unable to find any pathological changes in a number of kidneys obtained from animals which showed heart lesions and which had been previously infused, and also from animals which showed heart lesions but which had not been previously infused. We were unable to note any change in the glomeruli, renal epithelium or in the interstitial tissue. We also examined the urine from some animals with heart lesions and found it free of albumin. That this diminution of urine is dependent upon the cardiac lesions seems certain; as a provisional explanation, we may state that on account of the failure of the injured heart to respond to the increased demands upon it (the increased demand for work being due to the over-filling of the vascular system) the blood pressure falls, the lowering of the blood pressure being followed by a decrease in the amount of urine.

Upon ascitic and intestinal fluid the cardiac lesions have but little influence, the results obtained in normal animals and in animals showing a cardiac lesion varying but slightly; in non-nephrectomized animals infused with sodium chloride solutions, the differences are negligible; in non-nephrectomized animals treated with mixed sodium chloride and calcium chloride solutions the differences are slightly more marked but within the limits of spontaneous variation. We find in this series of non-nephrectomized animals which received mixtures of sodium chloride and calcium chloride solutions that the ascites is a little more increased and the intestinal

fluid more diminished than in normal rabbits infused with mixed sodium chloride and calcium chloride solutions in a larger series of control experiments. But we must not attach too much importance to these slight variations, especially as we have here to deal only with a relatively small series of six experiments and as we find similar figures in a small series of normal animals infused with mixed sodium chloride and calcium chloride solutions.

In nephrectomized animals infused with 0.85 per cent. sodium chloride solutions there are some differences, if we compare the animals with heart lesions with the normal animals of our earlier experiments, but the differences are less noticeable, indeed, practically negligible when we compare the series of animals with heart lesions with the newer series of normal animals, and since these last experiments were done at the same season as were the experiments on animals with heart lesions, these two series are more truly comparable. The experimental heart lesions influence therefore very markedly the secretion of urine, but leave the ascites and intestinal fluid almost unchanged.

In these series of experiments the occurrence of edema of the lung was noted more frequently than in normal animals. Thus, in normal animals which received sodium chloride solutions we found edema of the lung in 25 per cent. of the cases; among twenty-four animals which showed heart lesions, eight (33 per cent.) showed edema of the lung. In animals showing heart lesions which received mixed sodium chloride and calcium chloride solutions, five out of seven animals (70 per cent.) developed edema of the lung as compared with 44 per cent. of the normal animals infused with mixed sodium chloride and calcium chloride solutions. Here again we see that calcium chloride increases the frequency and severity of the edema of the lung and it seems probable that animals with myocarditic lesions are more liable to develop edema of the lung than are normal animals.

Of the various experimental factors used in our investigations, namely, addition of calcium chloride and of adrenalin to sodium chloride solutions, and myocarditic lesions, each affects the production of urine, intestinal fluid and peritoneal transudate in a specific way. Thus, calcium chloride diminishes markedly the amount of

urine, it also decreases the intestinal fluid and increases the peritoneal transudation. Myocarditic lesions decrease the urine and leave the intestinal fluid and peritoneal transudate almost unchanged. Adrenalin increases the urine and peritoneal transudate but leaves unchanged or possibly decreases somewhat the intestinal fluid. These results are represented in the following tables:

Comparative Influence of Various Experimental Factors on the Production of Urine, Intestinal Fluid and Peritoneal Transudate.

Standardized to Figures of the Sodium Chloride Series. (— decreased; + increased; = unchanged; (?) questionable.)

Experimental Condition.	Urine.	Intestinal Fluid.	Peritoneal Transudate.
Calcium chloride	—	—	+
Adrenalin	+	—(?)	+
Calcium chloride + adrenalin	—	—	+
Nephrectomy		+	+
Myocarditic lesion	—	=	=
Calcium chloride+myocarditic lesion.	—	—	+

Standardized to Figures of the Sodium Chloride and Calcium Chloride Series.

Experimental Condition.	Urine.	Intestinal Fluid.	Peritoneal Transudate.
Calcium chloride + adrenalin	+	—(?)	+
Calcium chloride+myocarditic lesion.	—	=	=

Our results indicate strongly that even granted that calcium chloride in the quantities used in our experiments diminishes the urine through its action on the circulation (a question which we intend to investigate experimentally) it does not exert its influence on the intestinal fluid and peritoneal transudate through the same mechanism. This conclusion is suggested by the fact that if the heart action has been weakened through a myocarditic lesion the urine may be more markedly decreased in quantity after infusion of sodium chloride solution than it is in normal animals under the influence of calcium chloride and that notwithstanding this decrease in the amount of urine in the animals with heart lesions, neither the intestinal fluid nor the peritoneal transudate are changed in the same direction as under the influence of calcium chloride.

SUMMARY.

1. Animals in which experimental myocarditis has been produced and which are infused with sodium chloride solutions show a marked decrease in the amount of the secretion of urine. This decrease is even more pronounced than that produced when calcium chloride is added to sodium chloride solution. The intestinal fluid which is markedly decreased, and the peritoneal transudate which is increased by calcium chloride are, on the other hand, not markedly influenced by myocarditic lesions. We may, therefore, conclude that calcium chloride does not exert its specific effect on the quantity of peritoneal fluid through a lowering of blood pressure.

2. Each of the three factors, namely, calcium chloride, adrenalin and myocarditic lesions affects the elimination of fluid through the kidneys, through the mucosa of the small intestine and through the endothelial lining of the peritoneal cavity in a specific way. The conditions influencing the elimination of fluids through these three surfaces seem, therefore, to be different in each case and characteristic for the cells lining these surfaces.

BIBLIOGRAPHY.

1. Lamy and Mayer, *Compt. rend. de la Soc. de biol.*, 1906, lxi, 102.
2. Porges and Pribram, *Arch. für exper. Path. und Pharm.*, 1908, lix, 30.
3. Fleisher, Hoyt and Loeb, *Jour. of Exper. Med.*, 1909, xi, 291.

STUDIES ON INTRACRANIAL PRESSURE.[1]

By J. A. E. EYSTER, M. T. BURROWS and C. R. ESSICK.

(*From the Physiological Laboratory of the Johns Hopkins University.*)

Plates XVI–XVIII.

The effects of a gradual increase of pressure within the skull cavity have been the subject of may physiological and clinical investigations. In the former, the effects upon the circulation, respiration, the motor phenomena of convulsions, reflex evacuations of the bladder and rectum and other attending phenomena, have been minutely analyzed by a number of observers. The more important of these investigations are reviewed in the works of Hill (1) and Cushing (2).

Cushing was the first to show clearly the exact effects of an increase of the intracranial pressure on the arterial blood pressure. This observer produced both local pressure on various portions of the brain and also general compression of the whole brain and cord by the inflow of fluid under pressure between the brain and brain membranes. He differentiated sharply between the effects produced by these two methods. Local compression of the brain produced symptoms which varied with the area upon which the pressure was directed, or with the portion or portions of the brain which suffer compression indirectly from displacement of the brain. In general compression, on the other hand, quite definite and constant effects upon the blood pressure and pulse rate were observed. These observations led to the formulation of a physiological law, namely that "an increase of intracranial tension occasions a rise of blood pressure which tends to find a level slightly above that of the pressure exerted upon the medulla."

One of the present writers in 1905 undertook a study of intracranial pressure with especial reference to its effects upon respiration and the development of a periodic rhythm, or as it is commonly known, Cheyne-Stokes respiration (3), a condition that had been observed by Cushing and other observers in conditions of increased

[1] Received for publication February 8, 1909.

intracranial pressure. The present communication embodies some observations made in these experiments as well as in numerous later experiments.

The main object in the present work has been to gain a clearer insight into the conditions accompanying the blood flow through the brain in states of increased intracranial pressure. With this object in view, several series of experiments were performed as follows: (1) Measurement of the lateral pressure in the circle of Willis under various degrees of intracranial pressure, by connecting the central end of the internal carotid artery with a manometer [method of Hürthle (4)]. (2) Measurement of the cerebral venous outflow under intracranial pressure (*a*) from the torcula and (*b*) from the emissary veins, a constant inflow of Ringer's fluid or of defibrinated blood being maintained through the peripheral (cerebral) ends of the internal carotids. (3) Injections of the brain vessels under different relations between the intracranial pressure and blood pressure.

Intracranial pressure was increased by the method previously employed by Cushing. "General compression" was used in all experiments. A trephine opening was made over some portion of the skull and into this was screwed an accurately fitting brass canula. The dura under the opening was excised and the cortex laid bare. The canula was connected with a reservoir of the fluid used for compression (defibrinated blood, undiluted or diluted with Ringer's fluid, Ringer's fluid, physiological saline solution), and the amount of pressure exerted measured by a mercury manometer connected with a side branch. The remaining details of the procedure differed in the different series of experiments and will be described separately.

THE EFFECTS UPON BLOOD PRESSURE AND PULSE RATE OF A GRADUAL INCREASE OF INTRACRANIAL PRESSURE.

It will be well to consider here somewhat briefly the main effects upon blood pressure and pulse rate that result from a gradual increase of intracranial pressure. It was observed by Cushing that experimentally degrees of intracranial compression less than the mean blood pressure have little or no effect upon blood pressure,

pulse rate or respiration. In several experiments we have maintained such low degrees of compression for considerable periods. In one experiment an intracranial pressure of 120 millimeters (40 millimeters lower than the blood pressure) was maintained for over five minutes with no change in the carotid blood pressure, pulse rate or respiration. If the animal is lightly anæsthetized, lower grades of intracranial pressure may cause changes in the respiration and pulse rate, probably due to stimulation of sensory nerves of the dura.

When the intracranial pressure is increased to a point approximately equal to mean arterial pressure as measured in the cardiac end of the carotid or femoral, the first effect is usually a slight rise of blood pressure. This is succeeded in six to ten seconds by a fall of longer or shorter duration, which in turn is followed by a rise of the blood pressure above the line of intracranial pressure. Following the first rise of blood pressure, two effects upon the circulation are usually seen, acting in opposite directions as regards blood pressure, namely, vaso-constriction and decrease in pulse rate. The latter at first usually exceeds the former and following the preliminary rise there is usually a fall of blood pressure, though even at this time the effect of the vaso-constriction may be seen on the blood pressure curve, the pressure not falling to so low a point as would ordinarily occur with the slow pulse rate, or even rising in the interval between the slow heart beats. The blood pressure may now rise to above the intracranial pressure and to a point higher than that before the intracranial pressure was increased, even though the pulse is still much slower than normal. With the rise of the blood pressure above the intracranial pressure the vagus inhibition passes off to a considerable extent, though the pulse usually remains permanently somewhat slower. If the intracranial pressure is still further raised, the changes in blood pressure and pulse rate may be duplicated in every detail, and within a short interval the blood pressure may assume a new and higher level somewhat above the intracranial pressure. In this way enormous systemic pressures may be attained—mean arterial pressures of 230 millimeters of mercury, or even more. If the intracranial pressure is lowered at any time, a rapid fall of blood pressure follows, and if the former reaches zero, conditions as regards blood pressure and

pulse rate return usually to that present before the intracranial pressure was increased.

The decrease in pulse rate, as was shown by Cushing, is due to a stimulation of the cardio-inhibitory center in the medulla, since it is not present after section of both vagi. It seems to be due to an anæmia of the center, since it occurs also from ligation of the cerebral arteries (Eyster) (3). Stimulation of the cardio-inhibitory center in intracranial pressure may apparently result from at least two causes—anæmia, and a considerable and fairly sudden increase of blood pressure. The former is probably the one acting when the intracranial pressure is raised, and is especially interesting when seen under the condition, which is not infrequent, of blood pressure rising and falling in the form of vaso-motor waves above and below the line of intracranial pressure. With each rise above the line of intracranial pressure the inhibition may partially or completely disappear, to return as the blood pressure falls below the intracranial pressure. The first of these relations between the intracranial and the blood pressure results in an anæmia of the medullary centers, the latter in a fairly normal blood supply. The second cause of vagus inhibition in intracranial pressure, a sudden increase of blood pressure, is seen not infrequently when the blood pressure is suddenly lowered. The result of the sudden lowering of the intracranial pressure from a point above blood pressure to a point considerably below is a sudden flow of blood to the brain and a rapid increase of pressure. This may produce marked inhibition (Plate XVI, Fig. 1). If the arterial pressure in the circle of Willis is recorded simultaneously, a sudden sharp rise in this pressure is seen to occur, and this rise is coincident with the inhibition.

The vaso-constriction, as was shown by Cushing from the fact that it was absent after section of the cord or paralysis of the medulla with cocaine, is also central in origin. Cushing exposed a loop of the small intestines and observed paling of the vessels with the rise of blood pressure produced by intracranial pressure. He therefore referred the rise to splanchnic constriction. We have repeated this observation a number of times and the paling is frequently perfectly definite. We have furthermore recorded the volume of an intestinal loop, of a kidney and of a limb, and have

found that all undergo constriction. This constriction is preceded however in all three of these parts by dilatation, as may be seen in the records (Plate XVI, Fig. 2, Plate XVII, Fig. 3). As has been stated above, the first effect of increasing the intracranial pressure is usually a small rise of blood pressure. This has been spoken of as the preliminary rise in order to distinguish it from the marked rise that occurs later and which is due to vaso-constriction. This preliminary rise of pressure is not due to vaso-constriction, since at this time the limb, intestine and kidney all show an increase in volume. The effect of increasing the intracranial pressure to above blood pressure is to render the whole brain anæmic with the exception of the basal arteries, as injections under such circumstances clearly show, and the systemic pressure is probably increased as a passive result of this reduction in size of the circulation and the forcing of blood from the cerebral circulation into the systemic circulation. The preliminary increase of volume of limb, kidney or intestine may occur in some cases when the preliminary rise of systemic pressure is practically absent (Fig. 2), and usually the degree of dilatation is much greater than that produced by similar rises of systemic pressure at other times. This suggests the possibility of an active process concerned in the dilatation. Figs. 2 and 3 are records of the intestinal and renal volume, respectively, in increased intracranial pressure.

The condition of blood pressure waves rising and falling above and below the line of intracranial pressure is seen in Fig. 3, and this record shows that the renal volume in this case shows corresponding variations, constricting as the blood pressure rises and dilating as it falls. Similar changes of volume associated with the waves of blood pressure occur also in the intestines and limb. The changes in blood pressure are therefore due to variations in the degree of vaso-constriction. It was suggested in a previous paper (3) that these waves probably arose from rhythmic stimulation of the constrictor center due to the periods of anæmia of the center that result when the blood pressure is below the intracranial pressure. The center thus stimulated responds with a rise in blood pressure. As the blood pressure rises above the intracranial pressure, the stimulus is much reduced, owing to the fact that the center is again supplied

with blood. The activity of the center is thus lessened and a fall of blood pressure follows. The following period of anæmia as the blood pressure falls below the intracranial pressure causes another period of stimulation and the same process is repeated.

In certain cases there is no reaction of the vasomotor center to the stimulus produced by the anæmia when the intracranial pressure exceeds the blood pressure. No rise of blood pressure follows, or there may be a slight rise too small to cause the blood pressure to rise above the intracranial pressure. In other cases the center may respond when the intracranial pressure is first raised, while an attempt to obtain a higher level by the still further increase of the intracranial pressure may be unsuccessful. If the reaction is not obtained and the intracranial pressure is maintained above the blood pressure for several minutes, death of the animal results from the medullary anæmia. The pressure falls quite rapidly and reaches a very low point, followed after some time by the stoppage of the heart. If the intracranial pressure is lowered shortly after this fall begins, and especially if artificial respiration is begun, recovery frequently follows. That this fall of pressure is actually due to a relaxation of the vaso-constrictor center is shown by recording simultaneously the intestinal volume. In such an experiment the intestine is seen to undergo rapid dilatation accompanying the fall of blood pressure.

THE LATERAL PRESSURE IN THE CIRCLE OF WILLIS UNDER INTRACRANIAL PRESSURE.

The method of measuring the lateral pressure in the circle of Willis was that used by Hürthle (4) and others. All the branches of the common carotid artery in the dog were tied on one side with the exception of the internal carotid. A canula was now placed in the common carotid facing toward the head and connected with a manometer. Arterial pressure from the central end of the same carotid was also recorded as well as the pressure exerted by the intracranial fluid. All these manometers recorded on the same surface and all were referable to the same base line.[2]

[2] It was found to be necessary, in order to record the pressure from such a small artery as the internal carotid in the dog, to use some fluid in the manometer

If the intracranial pressure is gradually raised from zero to a point equal to mean carotid pressure, there is a gradual rise of circle of Willis pressure, which may be great enough to cause the two pressures to become approximately equal. When the intracranial pressure is gradually raised to a point slightly below mean carotid pressure and then lowered, the pressure in the circle of Willis may be increased to a level approximately equal to that of carotid pressure with little or no effect on the latter. An example of this is seen in Chart 1. If on the other hand the intracranial pressure is

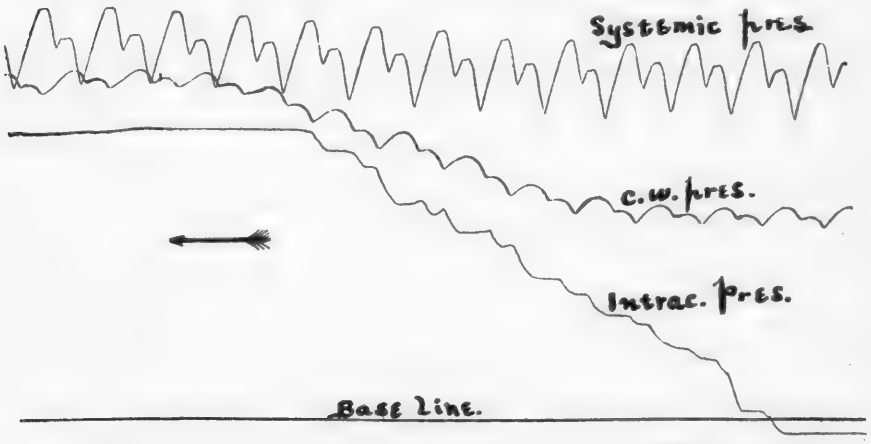


CHART 1. Increase of the lateral pressure in the circle of Willis with little change in the systemic (femoral) pressure from a gradual increase of intracranial pressure. The lowest line is the line of zero pressure for the intracranial, systemic and circle of Willis pressures. The kymographion was revolving at the rate of about 4.5 millimeters per second.

raised rapidly from zero to a point equal to carotid pressure, there is a rapid but small rise of pressure in the circle of Willis (Fig. 1). When the intracranial pressure rises above the carotid pressure, there begins a fall of circle of Willis pressure which, however, never reaches zero. The pulsations of the manometer in the circle of Willis are usually much reduced in size but do not disappear. If

and connections which does not have the marked vaso-constricting action of a saturated solution of sodium carbonate. Several of our first experiments were unsuccessful, due entirely to this factor. Sodium citrate (from 6 per cent. to 10 per cent.) proved entirely satisfactory.

the carotid pressure now rises, as is the usual result, the pressure in the circle of Willis rises very slowly until the carotid pressure becomes equal to the intracranial pressure and then rises much more rapidly as the carotid pressure becomes still greater. The pressure in the circle of Willis usually now becomes equal to carotid pressure. If the carotid pressure again falls below the intracranial pressure, or if the latter is raised above the carotid pressure, there is another fall of pressure in the circle of Willis, which again rises when the arterial pressure exceeds the intracranial pressure. If the intracranial pressure is lowered at any time, there is a sharp but short rise of pressure in the circle of Willis and then a fall accompanying the fall of carotid pressure. These various changes may be seen in Table I and in Plate XVI, Fig. 1, Chart 2 and Plate XVII, Fig. 4. Chart 2 shows the changes of pressure in the circle of Willis,

TABLE I.

No.	Intracranial pressure.	Femoral pressure.	Pressure in circle of Willis.	Pulse rate per min.	Remarks.
1	3	130	50	70	Intracranial pressure raised from
2	46	130	58	68	zero to above femoral pressure in
3	74	132	74	75	course of 54 seconds.
4	96	132	82		
5	120	146	81	73	"Preliminary increase" of femoral
6	146	145	60		pressure.
7	173	150	52	62	
8	173	91	22	20	Pulsations in C. W. small.
9	173	148	29	33	
10	173	160	32		9, 10 and 11 within 11 seconds.
11	173	173	34		Pulsations in circle of Willis in-
12	173	200	50	36	crease.
13	173	210	72		6 secs. after 12.
14	173	208	92		5 " " 13.
15	173	206	102		6 " " 14.
16	173	202	116	37	13 " " 15.
17	173	200	118		3 " " 16.
18	154	200	120		2 " " 17.
19	140	194	132		Rise of pressure in circle of Willis fol-
20	22	186	115	38	lowing fall of intracranial pressure.
21	15	170	90		19 to 21 within 15 secs.

carotid pressure and pulse rate that occurred in an experiment as the result of an increase of intracranial pressure.

These changes in the circle of Willis pressure under the influence of cerebral compression make it quite evident that an increase of intracranial pressure in the dog to a point above systemic pressure,

a condition which seems unquestionably to cause a complete absence of all circulation through the smaller vessels of the brain, does

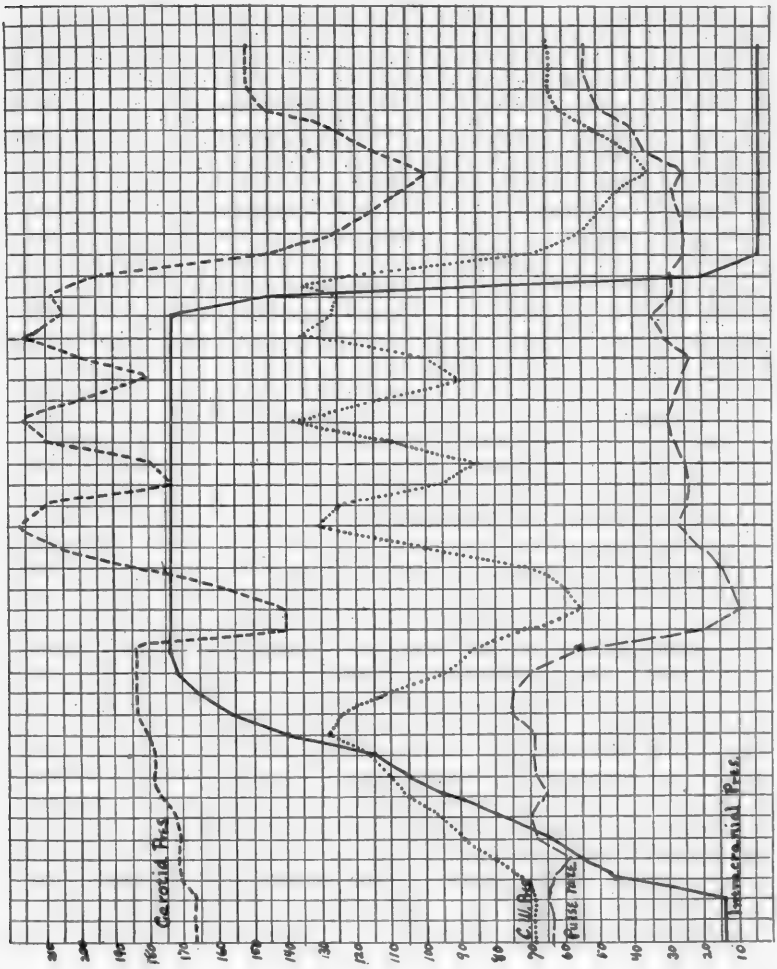


CHART 2. Chart from an experiment showing the changes of pressure in circle of Willis and in carotid after an increase of intracranial pressure. The abscissas represent time intervals of ten seconds, the ordinates pressure in millimeters of mercury and pulse rate per minute.

not cause a collapsed and bloodless condition of the circle of Willis nor a complete shutting off of this portion from the rest of the circulation. Direct observation of the cortical blood vessels through

a glass window fitted in the skull, at a time when the intracranial pressure is higher than the blood pressure, shows the presence of a complete anæmia of the capillaries and smaller vessels of the cortex. This observation was first made by Cushing and has been repeatedly confirmed by the present writers. Injections of the brain under these circumstances show also a complete anæmia. The circulation in the circle of Willis is however considerably affected under these conditions; the amount of blood that is received, as shown by the decrease in the size of the pulsations of the manometer connected with it, as well as by the pressure of this blood, are reduced. An interesting condition develops when the intracranial pressure is suddenly raised to a point far above carotid pressure. Under these circumstances there is a sudden sharp rise of pressure in the circle of Willis, which however is of small degree; there is little or no fall following, the pulsations cease entirely, and the whole picture shows that the circle is suddenly and completely clamped off from the rest of the circulation. It neither receives blood nor is there any flow of blood away from it either through arteries or veins.

The gradual increase of pressure in the circle of Willis when the intracranial pressure is slowly raised is probably due to a certain compression of the arteries as well as to a hindrance to the outflow through the veins. The fall when the intracranial pressure reaches carotid pressure may be explained by supposing that certain of the vessels entering the circle are clamped off, while the fact that the fall is not to zero and the pulsations do not entirely cease, is met by supposing that all of the arteries entering the circle are not thus occluded. With very high degrees of intracranial pressure, the results are such as would indicate a complete clamping off of the circle of Willis from the systemic circulation. The pressure does not fall but remains constant and there is an entire cessation of all pulsations of the circle of Willis. Injections of the brain under various degrees of intracranial pressure tend to support these suppositions in both cases, as will be shown later.

It is of some interest to compare the changes of pressure in the circle of Willis resulting from an increase of intracranial pressure with those caused by the actual ligation of the cerebral arteries. The results from one experiment of this kind are given in Table II.

TABLE II.[3]

Procedure.	Carotid Pressure.	Pressure in the Circle of Willis.	Amount of Fall of Pressure in Circle of Willis
Normal[4]	132	82	
Ligation of right carotid	140	64	18
Ligation of right vertebral	144	54	10
Ligation of left vertebral	154	22	32
Release of left vertebral	150	52	
Release of right vertebral	152	66	
Release of right carotid	140	82	

MEASUREMENT OF VENOUS OUTFLOW UNDER INTRACRANIAL PRESSURE.

The outflow from the cerebral vessels under artificial perfusion was measured in some experiments directly from the torcular, in others, from the emissary veins. The procedure may be best described by the following proctocol from an experiment:

Experiment of April 23, 1907.—Dog; morphia-ether anæsthesia; tracheotomy. Common carotids were dissected free to their bifurcation and the external carotid and all branches except the internal carotid ligated. Canulas were placed in the common carotids facing toward the brain. The two emissary veins were dissected down to their entrance into the skull, and canulas placed in them after ligating all branches between the point of entrance of the canulas and the emergence of the veins from the skull. The skull was trephined over the left temporal region and a brass canula screwed in and connected with the intracranial pressure apparatus. Ligatures were placed around the ascending aorta just above the heart and around the descending aorta near the diaphragm. All blood was washed out of the brain by running Ringer's fluid into the carotid canulas until the clear fluid emerged from the emissary veins. Death of the animal occurred at this point. The pressure of the entering perfusing fluid and of the exerted intracranial pressure were measured on the record by two mercury manometers. The outflow from the emissary veins was measured by a piston recorder which recorded on

[3] The pressure in the circle of Willis in this experiment falls from 82 to 22 millimeters of mercury following the ligation of the carotids, two vertebrals and subclavian arteries. As low a pressure as this is obtained in the circle of Willis in intracranial pressure only when there is very marked vagus inhibition with a considerable fall of systemic pressure, such as occurs in the experiment given in Table I. If pronounced slowing of the heart does not result, the fall of pressure in the circle of Willis from an increase of intracranial pressure above the systemic pressure is far less than that which occurs from ligation of the cerebral arteries. Thus, in Fig. 1, the fall was only from 40 to 30 millimeters in one case and from 40 to 36 in the other.

[4] The left carotid and the two subclavians had been previously ligated.

the same record. Measurement of the outflow was made under various relations between the intracranial and perfusion ("blood pressure") pressures.

The results from part of this experiment are given in Table III.

TABLE III.

The Perfusion Pressure Throughout the Experiment was Maintained at 96 Millimeters of Mercury. The Intracranial Pressure was Varied, as Shown in the Table.

No.	Intracranial pressure.	Outflow in c.c. per min.
1	30–166	3
2	172	2.2
3	172– 60	2.2
4	60– 40	2.7
5	40	2.7
6	30	3.0
7	30	3.3
8	18	5.3
9	130	5.0
10	190	2.1
11	190	0.3
12	18	1.5
13	18	2.7

In this experiment it is evident, with an intracranial pressure considerably above the perfusion pressure, that the outflow from the emissary veins is only slightly reduced below that present with an intracranial pressure considerably less than the perfusion pressure. Observation of the cerebral cortex as well as injections of the brain, show that under the former conditions the capillaries are completely occluded and free from blood or injection mass, not only on the surface of the brain but throughout the whole mass of the cerebrum, medulla and at least the greater part of the cord. It is therefore apparent, that with an artificial perfusion of the dog's brain through the internal carotid arteries, the whole capillary system may be entirely collapsed without decreasing to any considerable degree the outflow from the intracranial vessels as measured in the usual way from the emissary veins. With very high degrees of intracranial pressure relative to the blood pressure (190 millimeters in the preceding table) there is usually however a marked decrease in the amount of perfusing fluid flowing through the brain.

A number of experiments similar to the one described above have been performed with like results in all. In fact, the changes in

outflow as given in the table are greater than those usually obtained. The experiments in which the outflow was measured from the torcula gave similar results. In all experiments an increase of intracranial above the perfusion pressure produced none or an inconsiderable change in the outflow until the former pressure was approximately double the perfusion pressure.

A certain amount of the fluid used to increase the intracranial pressure escapes by way of the cerebral veins, and it is probable that in certain experiments this escape is sufficient to mask in some degree the decrease in flow through the brain that results from an increase of the intracranial pressure to above blood pressure. This loss of intracranial fluid is greater in some cases than in others, and its effects in extreme cases has been shown by the fact that a greater outflow from the emissary veins or the torcula occured when the intracranial pressure was above the perfusion pressure than when it was below it. The fact however that in many experiments there is no marked leakage of the intracranial fluid has been shown by a measurement of the outflow under intracranial pressure alone, in other cases by a direct measurement of the intracranial fluid lost during the course of an experiment, and in one experiment by measuring the inflow of the perfusing fluid into the carotids as well as the outflow from the veins. In several experiments the fluid for increasing the intracranial pressure was contained in a burette. The burette was connected on one side with the canula in the skull and on the other with the mercury pressure apparatus. In these experiments the leakage of intracranial fluid was too small to suppose that it masked to any considerable degree the reduction of outflow when the intracranial pressure was above the perfusion pressure. The results from one of these experiments are given in Table IV, and it is seen that if allowance is made for the loss of intracranial fluid the reduction in perfusion that results from an increase of the intracranial pressure above the perfusion pressure is inconsiderable. In this experiment defibrinated pig's blood was used for the intracranial compression, defibrinated dog's blood for the perfusion. The outflow was measured by a piston recorder.

TABLE IV.

Intracranial pressure.	Perfusion pressure.	Reading of intracranial burette.	Loss of intracranial fluid in c.c.	Period in secs.	Outflow in c.c.	Outflow in c.c. per min.	Corrected outflow in c.c. per min.[5]	Percentage of reduction of corrected outflow.
0	125	33.4		79	2.6	1.97		
140	125	33.4		176	5.2	1.71	1.61	18 per cent.
0	125	33.6	0.2	87	2.8	1.92		
0	165	34		115	2.5	1.30		
190	165	34		163	5.0	1.80	1.25	3 per cent.
0	165	35.6	1.6	107	3.8	2.10		
180	165	35.6		132	4.7	2.13	1.80	14 per cent.
0	165	36.3	0.7	102	4.0	2.35		
0	165	36.3		120	4.7	2.37		
160	0	36.3		50	0.165	0.20		
160	125	36.6	0.3	125	4.25	2.05		
0	125			92	4.1	2.67		

In one experiment the inflow of the perfusion fluid into the internal carotids was measured as well as the outflow. The results are given in Table V.

TABLE V.

Intracranial pressure.	Perfusion pressure.	Inflow into internal carotids in c c. per min.	Outflow from emissary veins in c.c. per min.
0	120	3.6	1.363
160	120	2.6	1.363
0	120	3.1	1.090
0	120	3.2	1.090
160	120	2.2	1.070
0	120		
0	120	2.8	0.818
150	120	2.0	0.818
0	120	2.7	0.800

An increase of the intracranial pressure to a point 40 millimeters above the perfusion pressure, a condition which, as shown by direct observation of the cortex and by injection, causes a complete occlusion and bloodless condition of all the capillaries of the brain,

[5] The loss of intracranial fluid during the period is subtracted from the total outflow and the result reduced to outflow in cubic centimeters per minute. Thus, when the intracranial pressure was increased to 190 millimeters, there was a loss of 1.6 c.c. of intracranial fluid in 163 seconds. The total outflow during this period was 5.0 c.c.; 1.6 subtracted from 5 gives a corrected outflow of 3.4 c.c. in 163 seconds or 1.25 c.c. per minute.

results in only a moderate decrease of the flow into the internal carotid arteries and of the outflow from the emissary veins. The outflow shows even less changes, a part being masked probably by a small leakage of the intracranial fluid.

Discussion of Results.—It seems quite evident from the results of the experiments described in the preceding pages that the method of perfusion of the dog's brain that has been used frequently in investigations on the cerebral circulation is open to grave criticism, since the whole capillary area of the brain may be completely collapsed with only a relatively small decrease in either the outflow or inflow of the perfusing fluid. This method as ordinarily employed consists in measuring the outflow from the emissary veins; the inflow, if the perfusion is an artificial one, is directed into the peripheral end of one or both of the common carotid arteries. Even if care is taken, as in all our experiments, to ligate all branches of the common carotids other than the internal carotids, the outflow from the emissary veins gives little or no indication of the condition of the cerebral vessels. This method has been used especially in the investigation of cerebral vaso-motor nerves, and as would be expected from the considerations presented above, has given rise to various and conflicting results. When the cerebral arterioles and capillaries are completely collapsed under a higher intracranial pressure than perfusion pressure, the change in outflow from the emissary veins is very small, and the dimunition in caliber which they would suffer as a result of vaso-constriction would probably not affect the outflow to any appreciable degree, certainly not sufficiently to warrant a positive conclusion.

So far as we are aware, only one investigator of cerebral vaso-motors, namely, Wiggers, has realized the fact that the ordinary method of perfusion of the dog's brain is open to severe criticism because of the possibility of collateral branches and anastomotic connections. This observer therefore severed the head, removed the lower jaw and laid bare the basilar artery and circle of Willis by removing carefully the basal portion of the skull, and then ligated all branches except those passing to the brain. He was able to show quite marked changes in outflow caused by the action of drugs in the perfusing fluid. Adrenalin caused a decrease in the

outflow under these circumstances, and other drugs caused changes which indicate with considerable certainty the presence of vaso-motor nerves (5). Due to the rather extensive injury to structures lying near the base of the brain it has been difficult to employ this method for the study of cerebral vaso-motors by means other than the action of drugs. Wiggers has attempted, however, to show changes in the outflow occurring as an effect of stimulation of the sympathetic plexus on the carotid artery (6). His results, while suggestive of the action of vaso-motor nerves on the caliber of the blood vessels, are not entirely conclusive.

The same criticism applies to those experiments performed in the investigation of cerebral vaso-motor nerves in which the lateral pressure has been measured in the circle of Willis. The method as employed consists in the simultaneous measurement of the pressure in the circle of Willis by connecting a manometer with the peripheral end of the internal carotid artery and of the pressure in the systemic circulation. A rise of the former without corresponding change in the latter is regarded as evidence of constriction. It is quite evident that if there are several paths open to the flow, constriction in one of these will cause an increase of presssure proportionately to the degree of constriction and the importance of this path relative to the other paths. Now if that path, the changes in which it is desired to investigate, namely, the vascular system of the brain itself, may undergo total collapse, and yet the other paths be sufficient so that the total outflow is affected only in very small degree, it is hardly to be supposed that the ordinary degree of vaso-motor constriction or dilatation in this path would cause any appreciable change in the blood pressure in the afferent vessel which is common to this and other paths.

Our experimental results indicate therefore quite clearly that in perfusion of the dog's brain, the inflow being directed into the internal carotid arteries and the outflow measured from the emissary veins, the perfusing fluid passes in part through some other path or paths than that of the vascular system of the brain itself, and that this collateral path is sufficient to prevent marked changes in the blood vessels of the brain affecting the total outflow to any considerable degree. Our attention was now directed to a determination,

if possible, of these collateral paths and an attempt to obtain a complete or practically complete isolation of the brain circulation in the dog without the removal of the brain from its nervous connections or opening of the skull cavity. With this object in view we made injections and dissections of the head, both under normal conditions and under different relations between the intracranial and injection pressures. When the injection was made into the internal carotids with a mass, such as Berlin blue, that passes through the capillaries, at a time when the intracranial pressure exceeded the injection pressure, then, as under normal conditions, the mass emerged from the emissary veins and the lateral sinuses were found fully injected, although the capillaries of the brain were entirely uninjected. When a mass was used which does not pass through the capillaries, such as ultra-marine blue, under similar relations of intracranial and injection pressure to that above, it was observed that those branches of the common carotid artery besides the internal carotid, namely, the internal maxillary, posterior auricular and occipital, vessels which have no direct communication with the circle of Willis, were partially filled with injection mass. These vessels were ligated at their origin from the common carotid; their injection therefore must have occurred from a peripheral direction, and this is only explicable on the assumption that there is some collateral arterial connection, independent of capillaries and veins, between the internal carotid artery, on the one hand, and the internal maxillary, posterior auricular and other branches of the common carotid, on the other. This connection may be along the course of the internal carotid before it enters into the circle, or these arteries may form anastomotic connections with some one or other of the branches of the circle of Willis. In suitably injected preparations we traced the internal carotid artery carefully from its origin to its entrance into the circle, and found in all animals that we examined, five in number, a surprisingly large communication of this artery with the ophthalmic branch of the internal maxillary.[6] This branch

[6] In man the ophthalmic artery arises from the internal carotid artery within the skull. In the dog, it arises from the internal maxillary artery after its exit from the pterygoid canal. It breaks up into branches to the muscles, lachrymal gland and eye, and gives rise to a branch which anastomoses with the artery of the corpus callosum.

is described by Ellenberger and Baum (7) as follows: "The internal carotid artery . . . having passed through the dura mater gives rise to two small rami which anastomose in the cranial cavity with the middle meningeal artery. One of these (the internal ophthalmic artery of Bellarminow) leaves the cranial cavity by the orbital fissure and anastomoses with the ophthalmic artery."[7]

In the animals that we have examined, this connection in three was single; in two it consisted of several small arteries running together. The vessel when single is usually larger than the internal carotid artery outside of the skull, and from its size, and from the fact that the internal carotid is approximately twice the size above the point from which this artery connects with it, it is rather to be regarded in the animals that we examined as a branch from the ophthalmic artery joining the internal carotid artery immediately before it enters the circle of Willis. The internal maxillary artery in the dog is therefore probably to be regarded as a cerebral artery equal or almost equal in importance to the internal carotid itself.

This arterial branch connecting the internal carotid and the ophthalmic division of the internal maxillary, although lying in the greater part of its length within the skull, occupies such a position that it is in great part protected from any but excessive degrees of intracranial pressure. It passes along the base of the skull on the floor of the middle cranial fossa. This fossa is unusually deep in the dog and across its upper border there is stretched a thick and tense plate of dura mater. In all but very high degrees of intracranial pressure it probably suffers no compression, as is shown by the fact that the artery is always found well filled in injections made with an intracranial pressure moderately increased above the injection pressure. The injection mass passes up the carotid to the point where this artery pierces the dura immediately below its entrance into the circle, but beyond the point where the connection is made with the anastomotic branch. If the intracranial pressure is only slightly higher than the injection pressure, the mass may pass into the circle of Willis, which will show a more or less complete injection. With an intracranial pressure much higher than

[7] A similar anastomosis between the internal carotid and middle meningeal arteries has been described by de Vries (*Archiv de Biol.*, 1904, xx, 375).

the injection pressure—two or three times as great—the pressure is such as to stretch the roof of membranes covering the middle cranial fossa, the anastomotic connection is compressed and is found free from injection. It is suggestive to note that it is only by such high degrees of intracranial pressure that any considerable change in the outflow in the perfusion experiments occurs, and that the point at which this occurs is quite sharply marked. The relations and connections of this artery are shown in Plate XVIII, Fig. 6.

The ophthalmic branch from the internal maxillary artery with which this anastomotic branch or branches connect, is quite a large artery and offers a path for the flow of blood or perfusing fluid which may be regarded as only slightly less than the path through the circle of Willis. Returning from the capillaries of the contents of the orbital cavity, the eye and the surrounding muscles and tissues, are the large ophthalmic veins, frequently forming a well-marked sinus at the base of the orbital cavity, and emptying into the cavernous sinus. It is to be further noted that in all of our injections with the exception of those in which the intracranial pressure was excessively high, the contents of the orbital cavity showed a complete injection.

The venous sinuses of the skull receive blood not only from the cerebral veins, but also from the veins of the pia mater, the dura mater, from the bone (diploic veins) and from the eye (ophthalmic veins) (8). The system of diploic veins and sinuses in the dog are of considerable importance in draining the cerebral hemispheres. The superior cerebral veins, the principal venous trunks formed from the veins of the cerebrum, according to the work of Cushing (2), after their entrance into the dura, divide into two main branches, one of which enters into the parietal foramen and opens into the lateral diploic sinus, the other passes within the dura and opens into the sagittal sinus. Cushing further states that because of their large size and connections the diploic sinuses must be regarded as important outflow paths, and that indeed a greater portion of the venous outflow must occur by this path than by way of the sagittal sinus. These offer furthermore a communication between the veins of the head outside of the cranial cavity and the cerebral sinuses. Another possible path besides that through the

optic circulation is therefore through the internal carotid to the internal maxillary, to the extra-cranial regions supplied by the branches of this artery, through the capillaries into the veins, to the diploic sinuses and thence by the connections above described to the lateral sinuses and emissary veins.

ATTEMPT TO OBTAIN AN ISOLATED CIRCULATION IN THE BRAIN.

We attempted in five animals to eliminate the accessory paths described above. Theoretically this should be obtained by ligation of the anastomotic branch connecting the ophthalmic and internal carotid arteries. The operation necessary is a very extensive one and attended with great technical difficulty, and in only one of these animals did we obtain results which would indicate that we had succeeded and in which it was evident at the autopsy that we had ligated this connection on both sides. The great difficulty was due to the fact that the nature of our experiments precluded opening any portion of the skull cavity. If this could be done, the operation would be much simplified. The artery to be ligated emerges from the skull at the base of the orbit through a small foramen and within a space of several millimeters joins the ophthalmic artery. In the one successful experiment we isolated these arteries on each side from any connection except the internal carotid. A description of this experiment follows.

Experiment of June 2, 1908.—Large dog; morphia-ether anæsthesia. The common carotid arteries were dissected up to the origin of the internal carotid arteries and all branches with the exception of the internal ligated. The emissary veins were exposed on each side. The lower jaw was completely removed and the zygomatic processes were resected on each side. The temporal muscles were peeled back from their origin, the outer portion of each orbital rim was removed and the base of the orbit was carefully dissected. Ligations were then made of the internal maxillary artery immediately before its entrance into the pterygoid canal, and of the ophthalmic vessels at the base of the orbit. This isolated not only the anastomosis between the ophthalmic and internal carotid, but also the middle meningeal, which arises from the internal maxillary immediately before this artery passes into the canal. Canulas were placed in the common carotid arteries facing toward the head and in the emissary veins. A trephine opening was made over the temporal region and a brass canula screwed in for the production of intracranial pressure. The dog was bled to death through the central end of one carotid; the blood was defibrinated, mixed with an equal volume of Ringer's fluid and used for perfusion. Undiluted defibrinated

dog's blood was used for the intracranial pressure. The inflow into the carotids, the loss of intracranial fluid and the outflow from the emissary veins were measured, the last by means of a piston recorder, the two former by means of burettes according to the method previously described.

The results from this experiment are given in Table VI, and in Plate XVII, Fig. 5.

TABLE VI.

No.	Perfusion pressure.	Intracranial pressure.	Inflow into carotids in c.c. per min.	Outflow from emissary veins in c.c. per min.	Loss of intracranial fluid.
2–3	130	0	1.6	1.0	
3–4	130	180	0.25	0.59	0
4–5	130	0	2.6	1.363	
5–6	130	170	0.3	0.545	0
6–7	130	0	1.35	0.954	
15–16	120	0	2.2	1.000	
16–17	120	180	0.5	0.681	0
17–18	0	180	0.0	0.454	0
18–19	120	0	2.3	0.612	

The amount of outflow in this experiment was reduced about fifty per cent. when the intracranial pressure was higher than the blood pressure. The reduction in the inflow through the carotids was much more marked, amounting to from one-fourth to one-eighth of the original flow in the examples given in the table. This would indicate that while the cerebral circulation was not completely isolated in this experiment, it was much more nearly so than in any of our previous experiments, and that the inflow, at least under these conditions, should show changes with any considerable change in the caliber of the cerebral arterioles. Of the other probable open paths, we have no indication from our experiments.

The outflow, as given in the table for the periods of compression, gives the average from the instant of increase of intracranial pressure until it was lowered. Reference to the figure will show that this does not give the whole state of affairs. The outflow at first is only slightly decreased; later a considerable decrease occurs and if the pressure is kept up for several minutes the outflow almost entirely ceases. Why the inflow should suffer a sudden and great reduction and a similar change in the outflow develop only gradually is not at present clear.

INJECTIONS OF THE BRAIN UNDER DIFFERENT RELATIONS BETWEEN THE INJECTION PRESSURE AND THE INTRACRANIAL PRESSURE.

Similar experiments to the ones we have performed have previoulsy been made by Cushing (2), and his results may be first summarized as follows:

Effect of Local Compression.—This study may be carried out in two ways: either the injection mass may be allowed to enter and then local pressure exerted greater than the blood pressure, both being maintained until hardening occurs, or the mass may be injected under a pressure lower than that of an already existing local compression. Both methods show absence of capillary injection in the region of compression, and if this compression is exerted over one hemisphere there is also absence of injection in the corresponding region of the opposite hemisphere. In the region of "injection anæmia," when the injection is made before the intracranial pressure is raised, the veins are filled with the injection mass, if the intracranial pressure is raised before injection, the veins are filled with blood. If the brain is hardened *in situ*, the point of stoppage of the injection in the latter case is seen where the veins leave the sulci and pass over the gyrus longitudinalis superior to the longitudinal sinus.

Effect of General Compression.—If the intracranial pressure is higher than the injection pressure a complete capillary "injection anæmia" occurs. The veins are filled either with blood or injection mass as in local compression according to the method of injection.

Our experiments were undertaken in this connection to determine, in the first place, the condition of the vessels when the general compression was less than the injection pressure, and the state of the vessels, especially in the medullary regions and interior of the brain mass when the intracranial pressure was higher than the injection pressure and was caused by the entrance of fluid over the cerebral cortex. Doubt has been expressed by several writers as to whether such procedure results in general compression of the whole brain, and though the experiments performed by Cushing and the present writers leave little question that "general compression" actually ensues, an anatomical proof has been desirable.

The *method of procedure* in these experiments was as follows: in all cases the intracranial pressure was produced by fluid and was exerted before injection was begun. The injection mass used was gelatin and Berlin blue. The carotids were exposed in the living anæsthetized animal and all branches but the internal carotids were ligated. The emissary veins were exposed and opened. The skull was trephined and connected with the intracranial pressure apparatus. The animal was bled to death from the central end of one carotid artery. The fluid employed to increase the intracranial pressure was warmed to body temperature to prevent setting of the gelatin before complete injection occurred. A ligature was drawn tightly around the lower part of the neck, the cerebral vessels washed out with warm Ringer's solution, the intracranial pressure raised to the desired point and injection begun. When the clear injection mass emerged from the emissary veins, the head of the animal was plunged into ice water, both the intracranial and injection pressures being maintained at their former constant level. After complete setting of the gelatin mass had occurred, the head was severed from the body and the brain removed or hardened *in situ*.

The results from these experiments may best be given by the description of certain typical specimens.

Specimen No. 3.—Injection pressure 160; intracranial pressure 100 millimeters of mercury. The circle of Willis and its connecting arteries and the veins around the base of the brain are normally injected. The whole lower and lateral surfaces of the brain show a normal capillary injection. The upper surface of the two hemispheres shows a good injection of the large arteries; the smaller arteries on exposed surfaces are only partially injected and in the more exposed surfaces there is an absence of capillary injection. Over the dorsal surface of the brain the veins in general are well injected; in certain of the more exposed situations, as in passing over convolutions, they are only partially injected or the injection shows breaks. The medulla shows a rich capillary injection. The interior of the brain mass shows a normal injection with the exception that for a distance of a few millimeters below the surfaces the injection as described above is not complete.

Specimen No. 4.—Injection pressure 100; intracranial pressure 150 millimeters of mercury. The circle of Willis and some of the larger arteries coming off from it are quite well injected. This is true especially of the anterior branches of the circle. The injection in these arteries ceases quite abruptly eight to ten millimeters after their origin from the circle. The smaller arteries and arterioles in this region are completely uninjected and the whole base of the brain with the exception of those vessels mentioned is completely colorless. The arteries, capillaries and veins throughout the rest of the specimen are completely uninjected. The medulla is entirely uninjected. The meninges show only faint traces of injection mass. The transverse sinus is incompletely injected; the lateral sinuses are full of injection mass. The eye is well injected.

Specimen No. 6.—Intracranial pressure 160; injection pressure 120 millimeters of mercury. The circle of Willis is fairly well injected. The rest of the brain, on the surface and in sections, shows an entire absence of injection mass. The medulla shows no trace of injection. About 12 cm. of the cervical cord shows entire absence of injection.

Specimen No. 13.—Injection pressure 100; intracranial pressure 200 millimeters of mercury. Entire absence of injection from any portion of the brain, superficial or deep. The circle of Willis is entirely free from injection.

The above experiments show that a condition of intracranial pressure produced by the general method and higher than the injection pressure, causes an "injection anæmia" of the capillary areas of the whole brain, including the medulla, the cervical cord and the interior of the brain mass. The larger arteries at the base of the brain are more or less protected from the compression when the intracranial pressure is not much higher than the injection pressure. The pressure is therefore transmitted not only throughout the cranial cavity but also through the brain substance. In these preparations the cerebral veins were uninjected, the sagittal sinus was partially injected and the lateral sinus and torcular were invariably full of the injection mass. In all cases the fluid used to increase the intracranial pressure entered over the temporal region of the cerebral cortex. In degrees of cerebral compression less than the injection pressure, the injection of the greater part of the brain was equal to that normally present. The regions directly beneath the entrance of the compressing fluid show a decrease in the capillary injection in certain areas and a collapse of certain veins in exposed situations. The local pressure seems to be therefore somewhat greater than the general pressure, but the fact seems to be well established that a considerable increase in the pressure of the intracranial fluid causes an increase of pressure upon the whole surface of the brain, the medulla, and at least the cervical portion of the spinal cord, and that this pressure is transmitted to the interior of the brain mass. If the intracranial pressure is greater than the blood pressure, complete anæmia of the brain occurs, both superficial and deep.

CONCLUSIONS.

1. The results of this work form a confirmation of the earlier work of Cushing in all details investigated.

2. The increase of blood pressure that results from an increase of the intracranial pressure above the blood pressure is due in the dog to a vaso-constriction of the vessels of the intestine, the kidney and the limbs. Preceding this constriction there is dilatation.

3. The anastomotic connection between the internal carotid artery within the skull and the ophthalmic branch of the internal maxillary is of a size in the dog approximately equal to the internal carotid outside of the skull. The anatomical relations are such as to indicate that the internal maxillary artery in this animal is a cerebral artery equal in importance to the internal carotid. The anastomotic connection because of its position within the cranial cavity is unaffected except by extreme grades of intracranial compression.

4. The ordinary method of artificial perfusion of the dog's brain as used in physiological investigation appears from this work to be of no value, since there is at least one important path open besides that through the vessels of the brain.

5. The increase of the intracranial pressure above the blood pressure leads to a complete anæmia, superficial and deep, of the blood vessels of the brain. If the intracranial pressure is not greatly increased above the blood pressure, the circle of Willis and some of the larger arterial connections at the base of the brain are more or less well injected. An intracranial pressure somewhat below blood pressure leads apparently to a certain degree of anæmia directly beneath the point of entrance of the intracranial fluid; the condition of the rest of the brain as regards blood supply is normal.

BIBLIOGRAPHY.

1. Hill, The Physiology and Pathology of the Cerebral Circulation, London, 1896; *Proc. of the Royal Soc.*, 1894, lv, 52.
2. Cushing, *Mitteil. aus den Grenz. der Med. und Chir.*, 1902, ix, 791. See also the *Amer. Jour. of the Med. Sciences*, 1902, cxxiv, 375; 1903, cxxxv, 1017; and *Johns Hopkins Hospital Bull.*, 1901, xii, 290.
3. Eyster, *Jour. of Exper. Med.*, 1906, viii, 565.
4. Hürthle, *Archiv f. d. gesammt. Physiol.*, 1899, xliv, 561.
5. Wiggers, *Amer. Jour. of Physiol.*, 1907–08, xx, 206.
6. Wiggers, *Amer. Jour. of Physiol.*, 1908, xxi, 454.
7. Ellenberger and Baum, Anat. descrip. et topograph. du chien, Paris, 1894, p. 378.
8. Ellenberger and Baum, *l. c.*, p. 448.

EXPLANATION OF PLATES XVI, XVII AND XVIII.

FIG. 1. Vagus inhibition from sudden lowering of the intracranial pressure. The lowest line is the line of zero pressure for the intracranial pressure, the lateral pressure in the circle of Willis and the pressure in the central end of the common carotid artery. The time is in intervals of one sceond.

FIG. 2. Record of the volume of an intestinal loop during an increase of intracranial pressure. The lowest line is the line of zero pressure for the carotid and intracranial pressures, and also records the time in intervals of one second. The numbers refer to corresponding points on the different curves.

FIG. 3. Record of volume of kidney during an increase of intracranial pressure. The record shows variations in volume of the kidney accompanying the vaso-motor waves of blood pressure. The numbers refer to corresponding points. Time in intervals of one second.

FIG. 4. The circle of Willis and carotid pressures during an increase of intracranial pressure. The record is to be interpreted as in Fig. 7.

FIG. 5. Measurement of the venous outflow from the emissary veins in the dog under intracranial pressure after ligation of the arterial connection between the internal carotid and ophthalmic arteries. The upper line represents the outflow as measured by a piston recorder; the lower line, the time in intervals of one second.

FIG. 6. From a dissection of the arteries in the region of the middle cranial fossa in the dog. The internal carotid artery is seen entering the skull and joined by the artery connecting it with the ophthalmic artery. This connecting artery and the internal carotid lie in the greater part of their course deep in the fossa and are protected by a covering of the brain membranes, on top of which is the circle of Willis. The internal carotid is seen piercing these membranes just before it enters the circle of Willis.

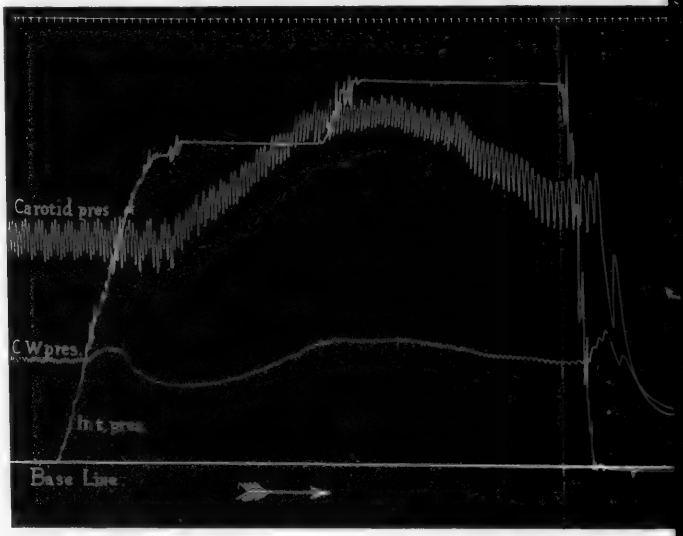

Carotid pres
C W pres.
Int. pres.
Base Line

FIG. 1.

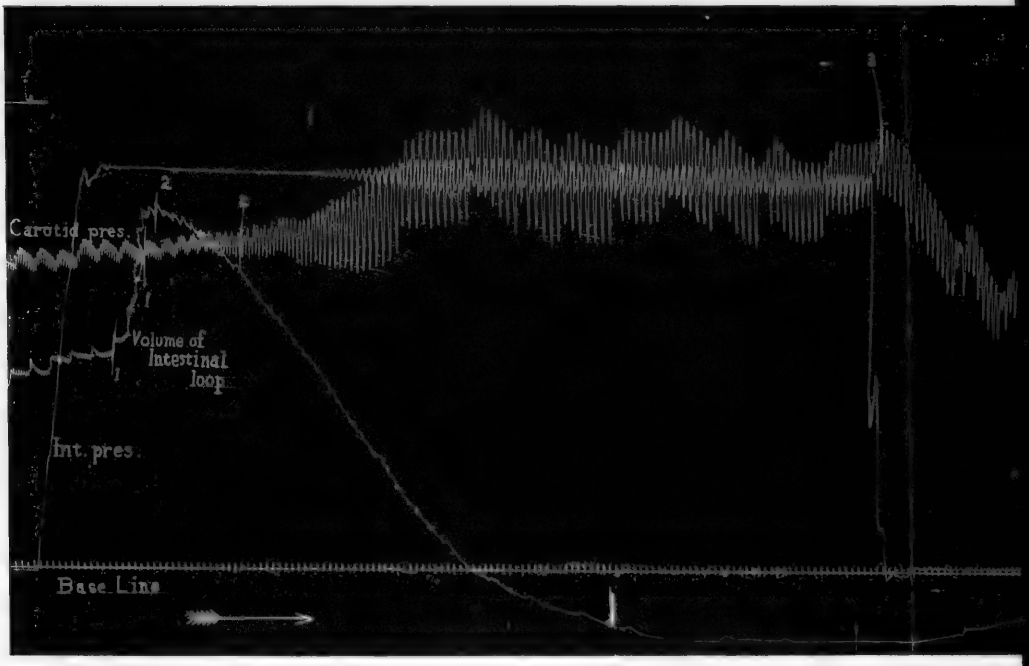
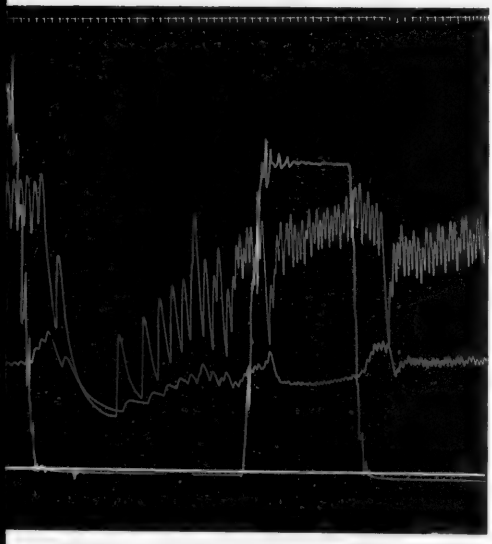
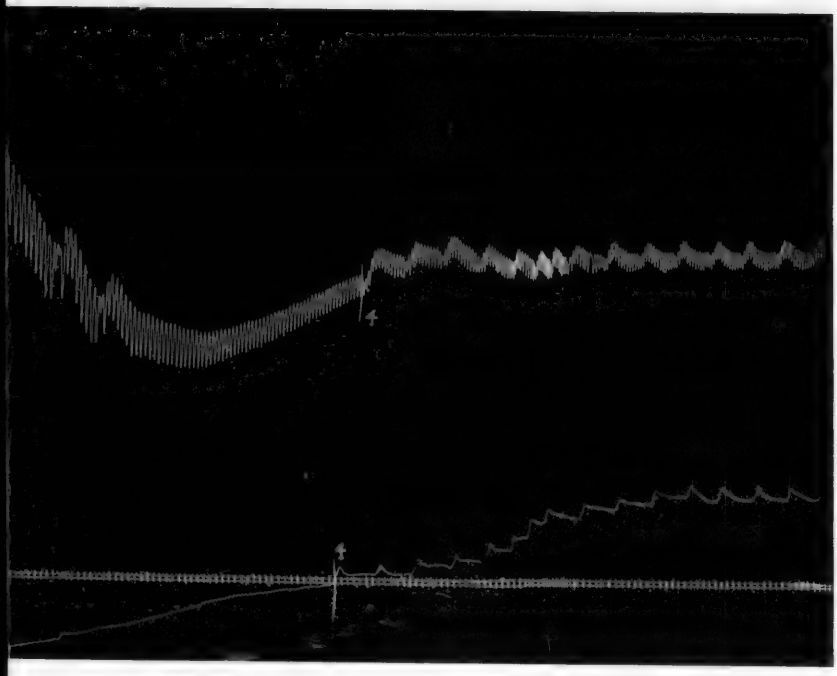

2
Carotid pres.
1
Volume of
Intestinal
loop
Int. pres.
Base Line

FIG. 2.

PLATE XVI.





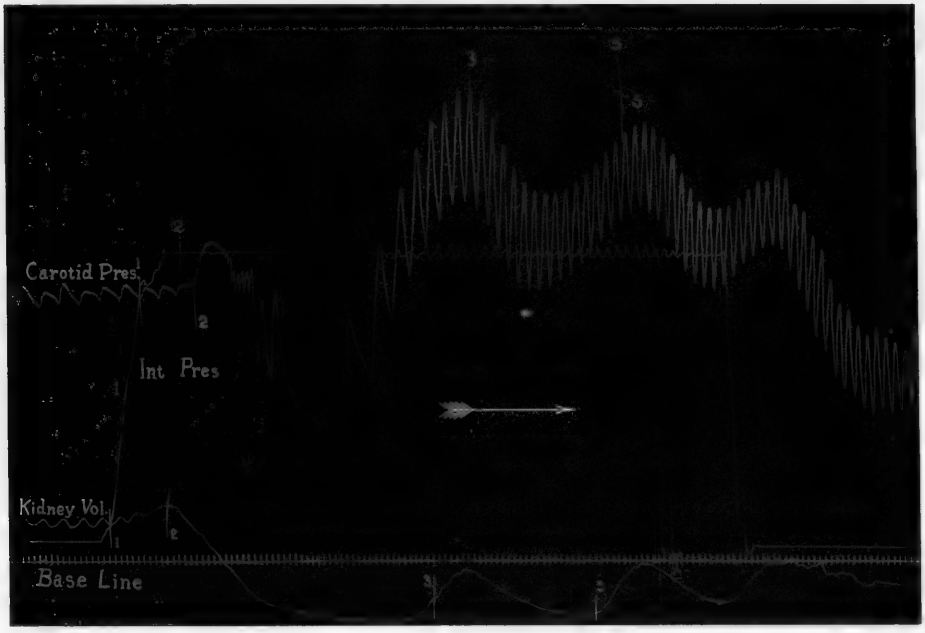
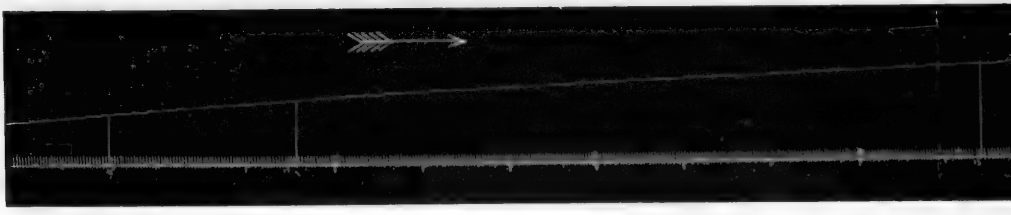


FIG. 3.



2 3 4

FIG. 5.

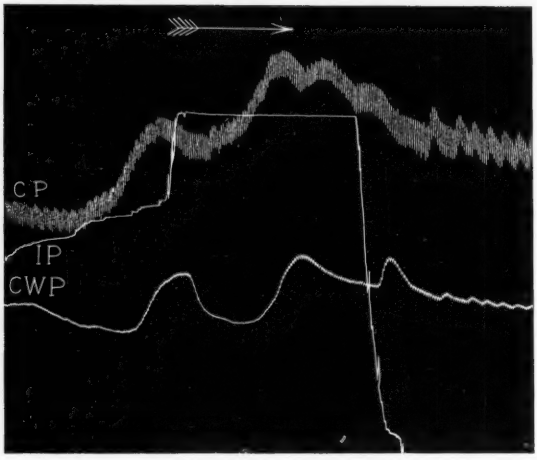
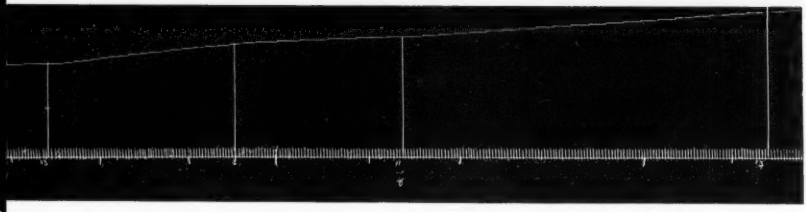


FIG. 4.



4 5 6

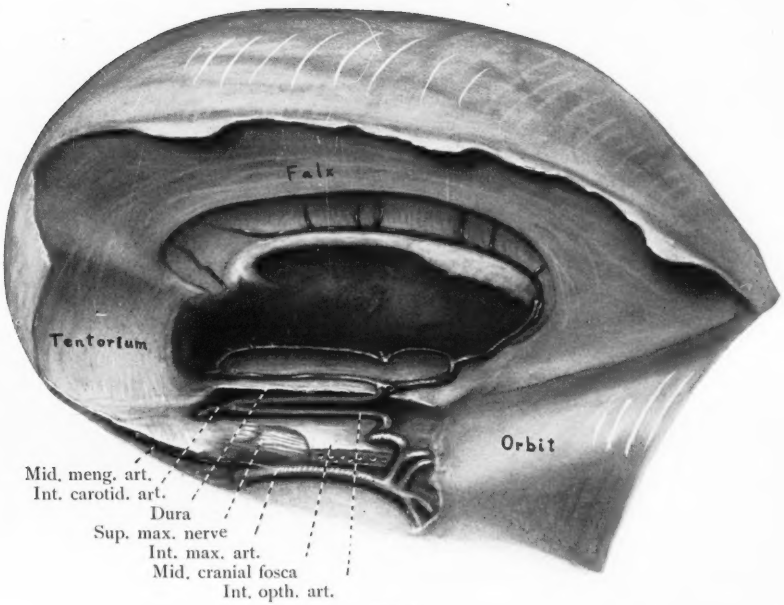

Falx
Tentorium
Orbit
Mid. meng. art.
Int. carotid. art.
Dura
Sup. max. nerve
Int. max. art.
Mid. cranial fosca
Int. opth. art.

FIG. 6.